Voltammetric Assays and Behavior of Norfloxacin And Nalidixic Acid Antibacterial Drugs

by

Ali Lounici

A Thesis Presented to the

FACULTY OF THE COLLEGE OF GRADUATE STUDIES

KING FAHD UNIVERSITY OF PETROLEUM & MINERALS

DHAHRAN, SAUDI ARABIA

In Partial Fulfillment of the
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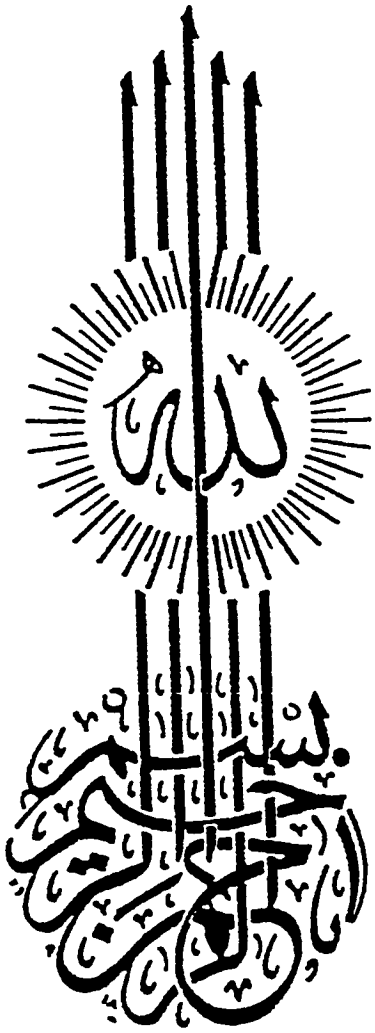
الله
بسم الله الرحمن الرحيم

Dedicated

to

my Parents, Brothers and Sisters

ACKNOWLEDGEMENT

Praise and gratitude be to ALLAH, THE ALMIGHTY, the creator and sustainer of the universe; with whose gracious help it was possible to accomplish this work. And peace be upon Prophet Mohammed, his progeny and faithful companions.

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THESIS ABSTRACT

NAME OF STUDENT : ALI LOUNICI

TITLE OF STUDY : VOLTAMMETRIC ASSAYS AND BEHAVIOR OF NORFLOXACIN AND NALIDIXIC ACID ANTIBACTERIAL DRUGS

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This thesis describes the work done on the voltammetric assays, physicochemical behavior, and mechanistic studies of norfloxacin and nalidixic and antibacterial drugs at the dropping mercury electrode (DME) and at the hanging mercury drop electrode (HMDE).

Easy, precise, accurate and fast analytical procedures have been developed for the quantitative determination of norfloxacin and nalidixic acid in formulated tablets using differential pulse polarography (DPP), linear scan voltammetry (LSV) and adsorptive striping voltammetry (AdSV). Good working ranges and recoveries with small standard deviations were obtained in the determination of both antibacterials using DPP and LSV techniques with a detection limit of 1 ppm (1 mg/l). Determination of ultra-trace of norfloxacin was possible using the AdSV with a detection limit of 0.31 ppt (0.31 ng/l).

The voltammetric behavior and mechanistic study of norfloxacin and nalidixic acid were investigated. Norfloxacin was found to be reduced in one electron step in strongly acidic and in alkaline solution. However, it is reduced in 2 one electron steps in slightly acidic to slightly alkaline solutions (pH 5.5 - 8.0). The two main reduction waves were found to be irreversible and diffusion controlled with scan rates > 20 mV/sec. However, the other smaller waves showed different degree of reversibility depending on the scan rate and were found to be adsorption controlled.

Nalidixic acid is found to be reduced in 2 one electron steps in strongly acidic medium and in slightly acidic to slightly alkaline solutions. However, it is reduced in one electron only in alkaline solutions. Its reduction processes were found to be completely irreversible and diffusion controlled.

MASTER OF SCIENCE DEGREE

KING FAHD UNIVERSITY OF PETROLEUM AND MINERALS
DHAHRAN, SAUDI ARABIA

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خلاصـــة الرسالـــة

اسـم الـطـالـب : علـي لونيـسـي

عـنـوان الدراسـة : تعيين و دراسة خواص عقاري الكينولون " نورفلوكساسين و حمض النـاليديكسيك" المضادين للبكـتيريا بالطرق الفولتامترية

التخصـــــص : كيمـيـاء تـحـلـيـلـيـة

تاريـخ الشهـادة : يـونـيـو ١٩٩٣

تصـف هذه الأطروحـة التحـاليل الفولتامتريـة، آليـة التفـاعل الكهروكيميـائي و الخـواص الفيزيوكيميائيـة لكل مـن النورفلوكساسين و حمض الناليديكسيك باستخدام قطب الزئبق القطري و قطرة الزئبق المعلقة.

لقد تم تطوير طرق تحليل كهروكيميائية جديدة، سهلة و سريعة، دقيقة و صحيحة للتقدير الكمـي لكل من العقـارين "نورفلوكساسين و حمض الناليديكسيك" فـي كل المنتوجـات التجاريـة الأولية و الجرعـات الدوائيـة باستخدام الطـرق الفولتامتريـة المختلفـة: البولاروغرافيـا النبظيـة التفاضليـة، فولتامتريـة المسـح الخطـي، و فولتامتريـة الاسـتخلاص الادمصاصي. و قد أمكن الحصول على مدى منحنيات قياسية عملية معقولـة واسترجاعات جيدة مـع انحرافـات قياسية صغيرة و ذلك عند تقدير كل من النورفلوكساسين و حمض الناليديكسيك باستخدام البولاروغرافيا النبظية التفاضلية و فولتامترية المسح الخطي، و أمكن الحصول على حد كشفي وصل الى جزء واحد في المليون (١ ملجرام/ لتر). و كذلك أمكن تقدير كميات ضئيلـة جدا من النورفلوكساسين وصـل فيهـا الحـد الكشـفي الـى ٠,٣١ جزء فـي التريليون (٠,٣١ نانوغرام/ لتر) باستخدام فولتامترية الاستخلاص الادمصاصي.

أجريت دراسة على التصرفات الفولتامترية و آلية التفاعل الكهروكيميائي للنورفلوكساسين و حمـض الناليديكسـيك، و قد وجد أن النورفلوكساسين يختزل في خطوة واحدة بانتقال الكترون واحد الى جزيئه في المحاليل عاليـة الحموضـة و المحاليل القلوية. و يختزل في خطوتين بانتقال في كل منهما الكترون واحد اليه في المحاليل قليلة الحموضـة و المحـاليل قليلة القلوية (الأس الهيدروجيني ٦,٥ – ٨,٠). و قد وجد أن موجتي الاختزال الرئيسيتين للنورفلوكساسين غير قابلـة للانعكاس و محكومة بالانتشـار عند استعمال سرعة مسح أكثر من ٢٠ مل فولت/الثانيـة. و قد أظهرت الموجـات الصغيرة الأخرى درجات مختلفة من الانعكاس معتمدة على سرعة المسح المستعملة و تبين أنها محكومة بالادمصاص.

وجد أن حمض الناليديكسيك يختزل في خطوتين ينتقل في كل منهما الكترون واحد الى جزيئه فـي المحـاليل عاليـة الحموضـة و المحاليل قليلة القلوية. و يختزل في خطوة واحدة ينتقل فيها الكترون واحد اليه في المحاليل القلويـة. و تبين أن موجات اختزاله غير قابلة للانعكاس نهائيا و أنها محكومة بالانتشار.

درجـة الماجسـتير في العلـوم

جامعة الملك فهد للبترول و المعادن

الظهران ، المملكـة العربيـة السعوديـة

يونيـو ١٩٩٣

CHAPTER ONE

INTRODUCTION

1.1 Voltammetry

Voltammetry is the study of current-voltage relationship at a working electrode. The most extensively used voltammetric method is polarography, in which the working electrode is a dropping mercury electrode (DME). In voltammetry an electron-transfer reaction can take place at a microelectrode if the potential is appropriate. The extent of this reaction is determined by the surface concentration of some electroactive species. Two major modes of mass transport from the bulk of solution to the electrode surface can be distinguished; namely, convection due to the motion of the solvent and supporting electrolyte, carrying the active species with it, and diffusion due to the movement of the active species through the solvent and the base electrolyte. Both of these modes are acting all the time that a current is passing through a cell, but usually one mode predominates. If the solution is stirred, mass transport takes place by convection (forced mass transport), but even here, diffusion contributes to the mass transport. It has been hypothesized a thin stationery layer of solution in contact with the electrode, that is unaffected by stirring; the mechanism of transport across this thin layer is by diffusion (1, 2). In the other case if there is no mechanical stirring (quiescent solution), the transport of active species occurs by diffusion or migration. In the case of diffusion, the motion is

caused by a gradient in chemical potential. Meanwhile, in the case of migration, the mass transport will be under the effect of an electrical potential gradient (3). Even in this circumstances, convection is never entirely absent, the residual effect is due to sources such as temperature changes and vibrations. In the presence of supporting electrolyte, migration current becomes negligible.

1.2 Polarography

Polarography is still one of the principal methods of analytical chemistry. Classical dc polarography is still used in chemical analysis, because of its simplicity and very cheap instrumentation. The operational parameters are easily adjusted on the instrument and thus information on the electrochemical behavior of the test substance, required for the development of an analytical method is obtained rapidly.

Among electrochemical methods of analysis, polarography has been widely recognized. The possibilities that polarography offered for the understanding of basic principles of both electrochemical processes and electroanalytical methods attracted the interest of numerous workers to that field, and resulted in the renaissance of electrochemistry (4).

Polarographic method of analysis is based on the study of electrolysis on a dropping mercury electrode (DME) and measurement of the resulting current-voltage curves. The shape of the curves obtained in electrolysis with the DME enables us to determine quantitatively some constituents of the solution and in some instances to detect the presence of a given

compound. Polarographic determinations can be performed at large dilutions and in small volumes of the electrolysed solution. The method can be described as economical in both time and material used. The curves recorded in polarographic electrolysis enables us to obtain information concerning the electrochemical properties of the substances involved, and of the chemical and electrochemical reactivity of these compounds.

1.2.1 Instrumentation for Polarography

The polarographic analyzer comprises four main components namely, ramp generator, a potentiostat, a cell containing three electrodes and a current amplifier and recorder (5). These components are shown in the schematic diagram in Fig. (1.1). The solution to be analyzed is transferred into the electrolysis vessel (cell) with a separated reference electrode, usually a calomel or silver/silver chloride electrode. The indicator electrode is a dropping mercury electrode. According to EG & G PAR instruments the slow outflow of mercury drops (0.5 - 5 sec) from the orifice is achieved by a mechanical system of control of drop size and time (Fig. 1.2). The drop is dislodged by means of a mechanical knocker. Voltage E is applied and controlled by the use of a potentiostat which controls the potential between the reference electrode and the DME. Thus the applied voltage is practically equal to the potential of the indicator DME.

The method can be illustrated when the applied voltage E is increased regularly noting the increase in the corresponding current i. A current-voltage curve known as polarographic wave is obtained at the end.

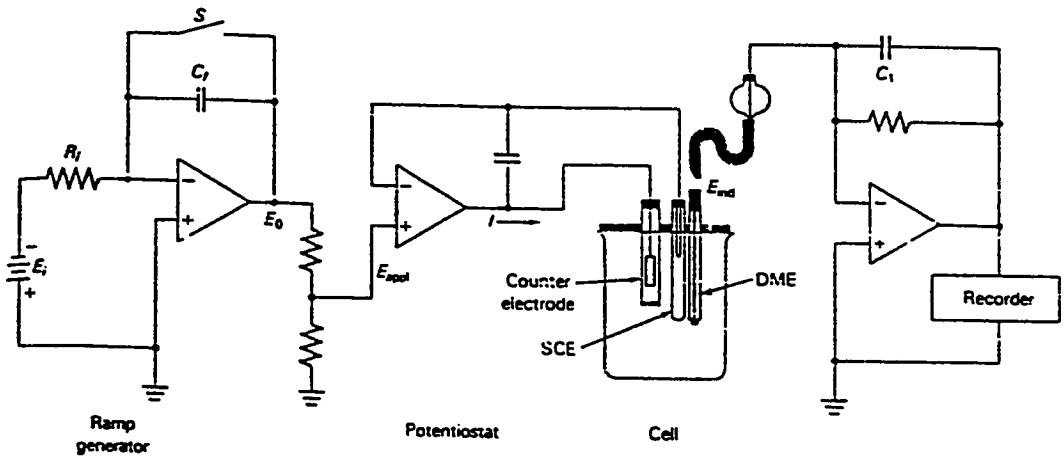


Figure 1.1 Schematic diagram showing the components of a modern, three-electrode polarograph and cell.

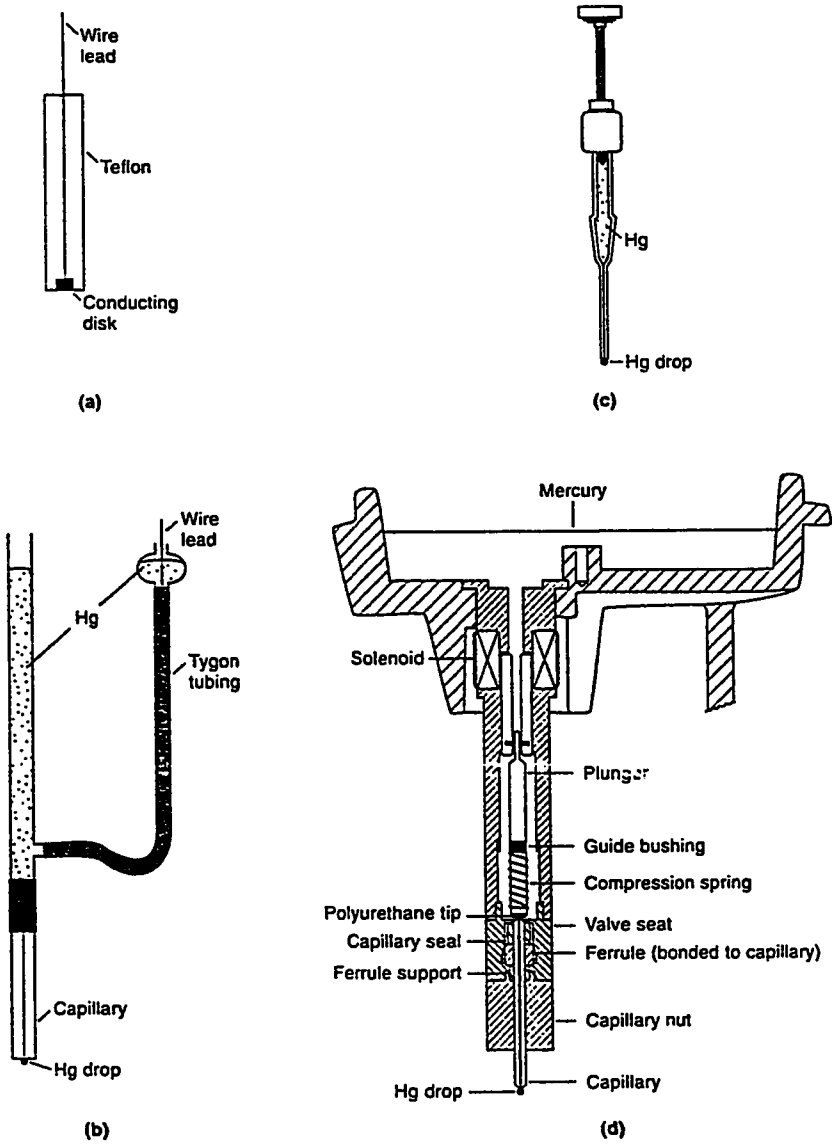


Figure 1.2 Some common types of microclectrodes; a) a disk electrode; b) a handing mercury drop electrode; c) a dropping mercury electrode; d) a static mercury dropping electrode.

This curve denotes by its potential the nature and by its height the concentration of the electrolysed species in the solution (Fig. 1.3).

In polarography it is a convention to denote as cathodic those currents, corresponding to a reduction process occurring above the galvanometer zero line. Currents flowing in the opposite direction, from oxidation process and causing deflections under the zero line, are called anodic currents. The potential, at which the current reaches the half value of the limiting current is called the "half-wave potential" $E_{1/2}$ (Fig. 1.3).

The $E_{1/2}$ value of the oxidation or reduction potential is a physical constant, in most instances practically independent of the concentration of the particular compound and characterizing quantitatively the electroactive compound.

1.3 Types of Polarographic Limiting Currents

1.3.1 Diffusion Currents

The most important type of polarographic limiting currents used for practical purposes are the diffusion limiting currents (6, 4). The diffusion current is a type of limiting current in which its magnitude is limited by the rate of diffusion of electroactive species from the bulk of solution to the surface of the mercury electrode. The constant value of the limiting diffusion current is given by the particular number of particles, reaching

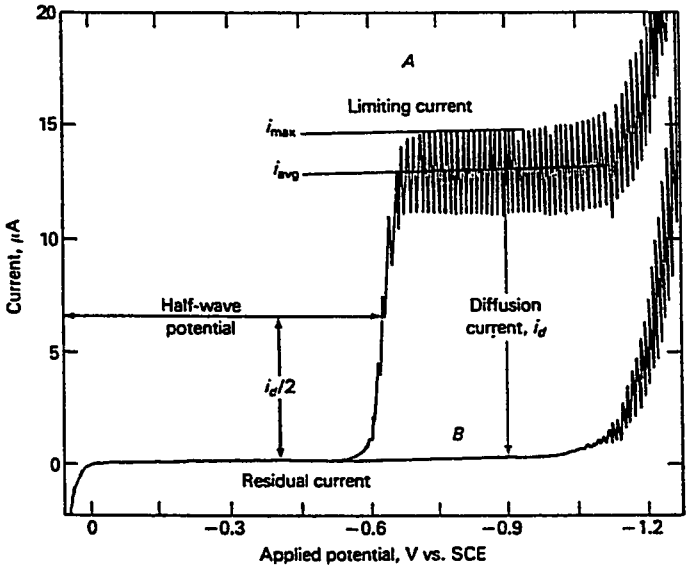


Figure 1.3 Polarograms for:
(A) a 1 M solution of HCl that is 5 x 10^{-4} M in Cd^{2+} and
(B) a 1 M solution of HCl. (From D. T. Sawyer and J.L. Roberts Jr., Experimental Electrochemistry for Chemists. New York: Wiley, 1974. Reprinted by permission of John Wiley and Sons, Inc.

the surface of the dropping electrode during the drop-time. The magnitude of the diffusion current (i_d) is given by the Ilkovic equation (3):

$$i_d = 0.627\, n\, F\, D^{1/2}\, m^{2/3}\, t^{1/6}\, .\, C$$

where n is the number of electrons involved in the rate determining step. F is the Faraday constant; i.e. a charge of 96500 coulombs. D is the diffusion coefficient (cm^2/sec) of the polarographically active species. m is the flow rate of mercury in (mg/sec) and t is the drop time in seconds. When variables other than the concentration are kept constants, the diffusion current may be given by the equation,

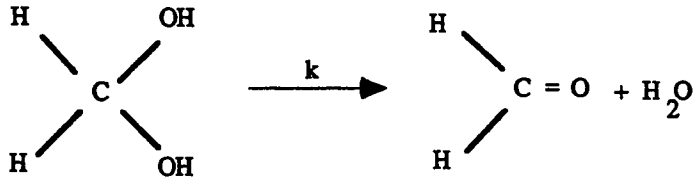
$$i_d = K.\, C$$

This equation shows a linear relationship between the diffusion current (i_d) and concentration C (in moles per litre) if the factor K remains constant. A rise in temperature of 1°C causes an increase in the height of the diffusion current by about 1.5 percent of the original value (7). To ensure the linear relationship between the diffusion current and concentration it is necessary to maintain the temperature and mercury pressure constant, and to use the same capillary for all curves compared for analytical purposes

1.3.2 Kinetic Currents

In some cases, an electroactive form other than the substance present in the bulk of solution is formed, by a chemical reaction in the neighbourhood of the electrode, and consequently undergoes reduction or oxidation at the DME. In such instances, when the rate of a chemical

process is slow enough to be the determining step of the electrode process, the term kinetic current (i_k) is used. These homogeneous chemical reactions may precede the electrochemical reaction and produce the electroactive species itself (CE mechanism). In other instances they may follow the electrochemical reaction thus, the product of the electrode process reacts to produce non-electroactive species (EC mechanism). An example of such currents occur in the reduction process of formaldehyde in aqueous solutions. In this case the nonreducible hydrated form predominates. The reducible dehydrated aldehydic form is formed from the hydrated form by a general acid-base catalyzed reaction:



The rate of this reaction k determines the height of the polarographic reduction wave, and since the rate is strongly influenced by changes in pH and temperature, the polarographic limiting current is also greatly dependent on the pH of the supporting electrolyte and the temperature of the electrolyzed solution (8).

1.3.3 Catalytic Currents

Principally, two types of polarographic currents are described as catalytic currents. In the first type, the limiting diffusion current of an electroactive substance is increased in the presence of a catalyst, which itself is either polarographically inactive or reduced at considerably more

negative potentials (9). In the second type, the polarographically inactive catalyst causes a shift in the reduction potential of the polarographically active substance towards more positive potentials.

Examples of the first type which belongs to the most precisely treated polarographic currents, are the reduction of Fe (III) or Ti (IV) in the presence of different oxidizing agents (6), such as hydroperoxide, hydroxylamine, chlorate etc. The waves measured are those corresponding to the reduction of the metallic ions to the lower valency state, and the limiting currents of these waves are increased in presence of oxidizing agents (owing to the regeneration of the reducible form from the product of the electrode process). The reaction proceeds as follows:

$$M^{n+} + e \underset{k\,[OX]}{\rightleftharpoons} M^{(n-1)+}$$

Where OX represents the oxidizing agents. The limiting value of such catalytic currents (also described as regeneration currents i_{reg}), is given by the following equation (8):

$$\frac{i_{reg}}{i_d} = 0.81\,(4.2\,k\,[OX]\,t)^{1/2}$$

This shows the dependence of the current on the concentration of the oxidizing agents.

The second type of catalytic currents is due to the presence of some substances which lower the hydrogen over voltage (8). In such instances,

the hydrogen ions are reduced at more positive potentials than those corresponding to the reduction of protons from strongly acidic or buffered solutions leading to the formation of a catalytic hydrogen wave. The hydrogen ion is reduced after interaction with the catalytically active substances, and in the course of the reaction the catalyst in the unprotonized form is regenerated. Thus, such catalytic waves are often many times higher than the corresponding diffusion current at the given concentration of the catalyst (9). Catalytic currents in most instances, are not a linear function of the catalyst concentration, and they reach a limiting value with the increasing concentration of the catalytically active substance.

1.3.4 Adsorption Currents

The polarographically active species, the product of the electrode reaction or some other components of the solution may be adsorbed at the DME surface. In such instances two waves may be observed on polarographic curves, one corresponds to the reduction (or oxidation) of the free form and the other for the adsorbed form (10). The adsorption current increases with the increasing concentration of the electroactive species, but only to a certain value, which is a function of the surface of the electrode. No further increase of current is observed with further increase of concentration, and, it is supposed that the surface of the electrode under such conditions is covered by a layer of the adsorbed substance. The adsorption current (i_d) is given by the equation (4):

$$i_d = 0.85\ n.\ F.\ Z.\ m^{2/3}\ t^{-1/3}$$

where Z is the number of moles of the substance adsorbed on 1 cm^2 of the

surface of the electrode. The other symbols are the same as in the Ilkovic equation. The adsorption energy causes a shift in the half-wave potentials to more positive or more negative values (4).

1.3.5 Other Types of Currents in Polarography

Charging current :

An electric double layer consists of one layer of charges surrounding the electrode surface and a second layer of opposite charges in the immediate vicinity of the electrode. This double layer behaves as a condenser. Thus, a certain current is needed to charge the electrode to the required potential. This charging current has a zero value in the case of stationary electrodes. With DME each drop must be charged to the required potential. Thus, there is a continuous passage of this current which is a function of the surface area growth of the mercury drop. This current is observed even in pure supporting electrolyte. It depends on the magnitude of the surface of the mercury drop; and is proportional to the height of the mercury column. It may be expressed by this equation

$$i_c = kV\, t^{-1/3}$$

where V is the potential during the drop lifetime which may be considered constant.

Migration current :

It is observed in addition to the diffusion current in solutions with

insufficient concentration of supporting electrolyte. This current is due to the movement or migration of ions in the electric field between the two electrodes. Thus, the mass transfer process of the electroactive species will be modified by migration. The magnitude of this current depends upon the transference number of the species. Addition of supporting electrolyte whose ions do not contribute to the electrode process will reduce the transference number of the electroactive species. Under normal conditions, in which the molar concentration of the supporting electrolyte is more than 100 times higher than that of the substance studied, this current is of negligible value (1, 8). The transference number of the electroactive species becomes practically zero.

Maxima:

Sharp or rounded rise of current in the form of a maximum is often observed on the convertional dc polarographic curves. These maxima are accompanied by motion of the the solution near the DME (2). This movement causes an increased transport of the electroactive substance to the surface of the electrode. All types of Maxima can be suppressed by gelation or some other surface active substance.

1.4 Voltammetric Methods Used in this Study

1.4.1 DC Polarography

DC polarography is considered one of the classical techniques used in electrochemistry. It is an electroanalytical technique in which the current flowing at a dropping mercury electrode (DME) is measured as a function

of potential. Important features of such a polarogram can be seen in Figure 1.4. The diffusion current is limited by the rate of arrival of the electroactive substance to the electrode surface. The value of $E_{1/2}$ is characteristic of the particular substance undergoing reaction at the electrode, and can be useful in the identification of solution constituents. However, for a particular substance, it is often a function of the solution conditions such as supporting electrolyte, pH and solvent system.

A reversible electrochemical reaction is one in which the electron transfer rate appears infinitely fast by the technique used for measurement. In polarography, electron-transfer rate constants of 2×10^{-2} cm/s and greater appear reversible. However, those with rate constants below 3×10^{-5} cm/sec appear totally irreversible (11). The current-potential data over the current step region of the polarogram can be used to determine the degree of reversibility of a particular reaction. The plot of E_{dme} versus the log term in the equation:

$$E = E_{1/2} - \frac{0.0591}{n} \log \frac{i}{i_d - i}$$

will have a slope of 59.1/n mV at 25°C for a reversible system (Fig. 1.5). Values greater than this indicate irreversibility. However, this test does not prove the reversibility of a given reaction. The intercept of this plot can also be used to obtain $E_{1/2}$ values. For irreversible systems the current-potential relationship is not simple and contain terms relating to the electron transfer characteristics of the reaction. In fact, the current voltage

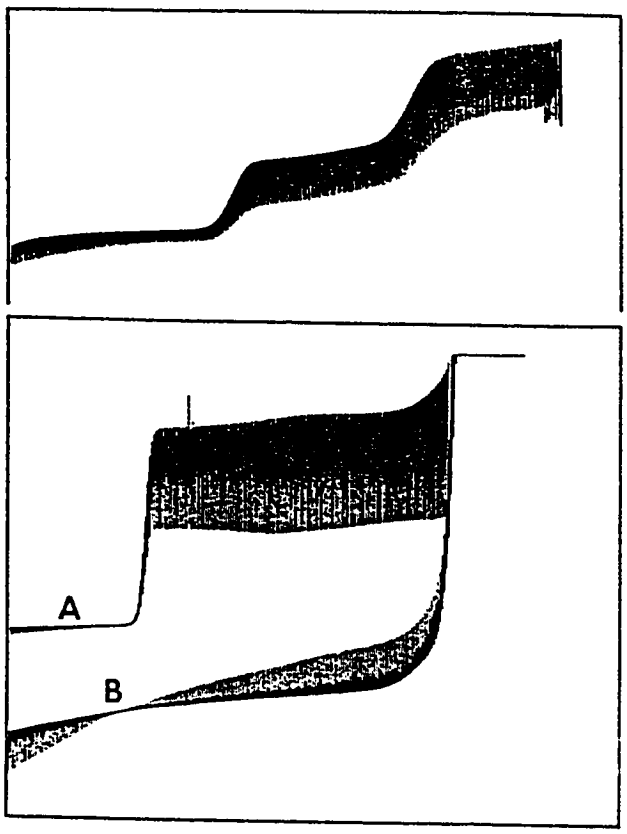


Figure 1.4 Features of a dc Polarogram.

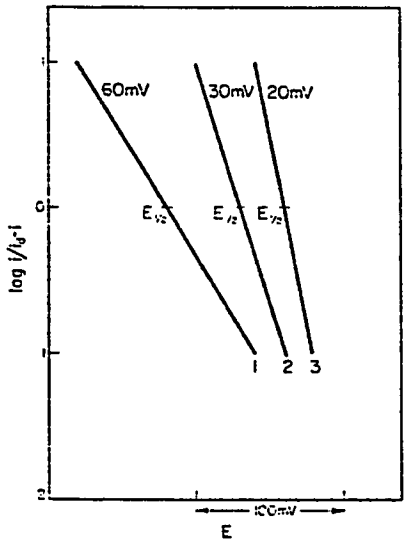


Figure 1.5 Logarithmic analysis of polarographic curves.
(1) n = 1; (2) n = 2; (3) n = 3 for t = 30°C.

relationship was given for the irreversible systems by the following equation,

$$E = E_{1/2} - \frac{0.0591}{\alpha n} \log \frac{i}{i_d - i}$$

where α is the charge transfer coefficient and its values lie in the range 0 to 1.

The advantage of polarography is that the mercury drop surface is continuously renewable thus eliminating any poisoning. A disadvantage is that each new drop that appears requires a double layer charging current which flows on the emergence of each new drop, thus limiting the analytical usefulness of the method.

1.4.2 Current-Sampled DC Polarography (TAST Polarography)

An improvement that has been made in classical polarography by introducing the sampled DC method involves measuring the polarographic current at the end of the mercury drop lifetime (12). This sampled current reflects the analytical contribution of the current better than in the case of DC polarography. Since the sharp double layer charging spikes present in ordinary DC polarography are eliminated and a clearer wave without the use of filters will be obtained as shown in Fig. 1.6.

The charging current decays with drop age according to $t^{-1/3}$, while the Faradaic current increases as $t^{1/6}$. By sampling the current just before the drop falls, the charging current is minimized meanwhile, the Faradaic current is maximized.

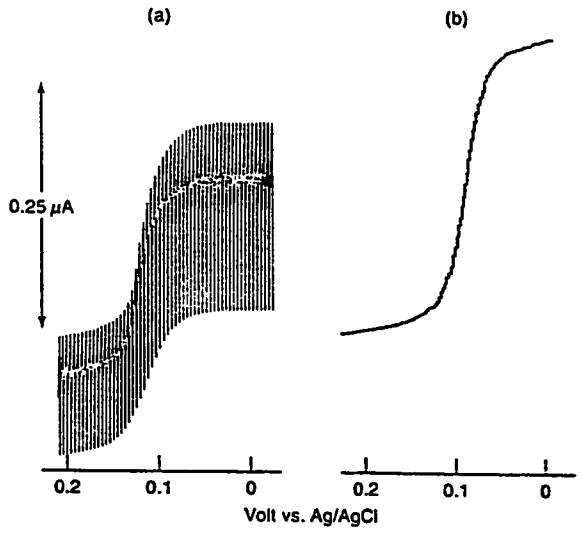


Figure 1.6 Comparison of classical (a) and current sampled (b) polarographic waves of 1 x 10^{-4} M solution of Cu^{2+} in 1M $NaNO_3$. (Reprinted with permission from A.M. Bond and D.R. Canterford, Anal. Chem., 1972, 44, 721-copyright 1972 American Chemical Society.

1.4.3 Differential Pulse Polarography (DPP)

It is one of the modern polarographic techniques. In this technique, a slightly more complicated waveform is applied within the drop lifetime where pulses of small amplitude (~50 mV) are applied on a regularly increasing ramp, Fig. 1.7(a). The current is sampled once just before the application of the pulse and again at the end of the pulse. The readout presented to the recorder is the difference between these two currents. Such a differential signal provides something approximating the derivative of the polarographic wave, and thus gives a peak-shaped curve Fig. 1.7(b). The peak position lies close to the half-wave potential $E_{1/2}$ found in dc polarography

$$E_p = E_{1/2} \pm \frac{\Delta E_p}{2}, \quad (\Delta E_p = E_p \text{ cathodic} - E_p \text{ anodic})$$

where for a reduction process the peak is shifted in a positive direction as the pulse amplitude increases (12). The magnitude of the signal obtained is strongly dependent on the amplitude of the pulse. The larger the pulse, the larger the signal obtained. However, a reduction in pulse amplitude permits the resolution of the fine details of the wave.

The differential technique turns out to be extremely sensitive, yielding large easily defined peaks for concentrations as low as 5 ppb or less (11). It is of extreme utility in analytical laboratories, since it is applicable to both inorganic and organic solutions and to both metal and organic analysis problems. In addition, the fact that determinations of

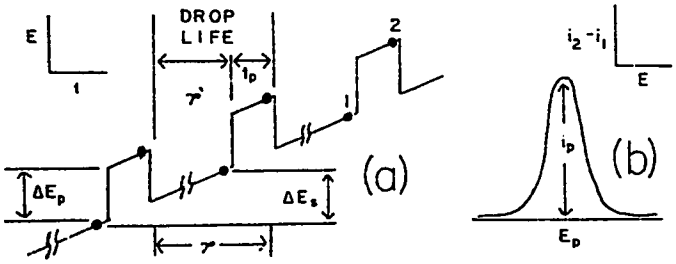


Figure 1.7 Differential pulse polarographic potential scan is shown in (a) and the resulting current peak is shown in (b). The peak is symmetrical for a reversible couple at a dropping mercury electrode.

more than one element can be accomplished simultaneously as long as the halfwave potentials of these elements are not too close. Moreover, pulse polarographic techniques offer a significant advantage in that they can be used with electrodes other than the dropping mercury electrode while still maintaining all the advantages of DME polarography. Langmuirian adsorption has been simulated and up to eight different metals have been determined simultaneously using standard addition method (13, 9).

1.4.4 Linear Sweep Voltammetry and Cyclic Voltammetry in Quiescent Solutions

In cyclic voltammetry (CV) the current is monitored during a triangular potential sweep resulting in plots of both cathodic and anodic current responses (Fig. 1.8). The technique is performed with a solid or with a hanging drop mercury electrode (HDME) where the analysis takes place on a single mercury drop. In the case of linear sweep voltammetry (LSV) at stationary electrodes, it involves monitoring current as a function of applied potential when a regularly varying potential is applied to the working electrode. However, the potential sweep is not cycled (Fig. 1.9). With both methods the solution is unstirred, and mass transport is the result of diffusion alone. Rapid scan rates, typically 50 mV/sec or faster, are used in CV and LSV analysis.

Cyclic voltammetry is a fast, convenient method for studying the kinetics of electrode processes. It is an extension of linear sweep voltammetry that employs repetitive voltage sweeps in alternate directions.

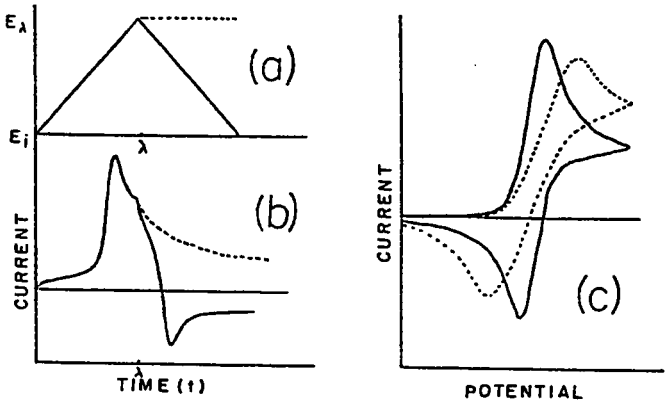


Figure 1.8 Cyclic voltametry (a) potential profile, (b) current time profile for a reversible system, and (c) current-potential profile for a reversible system (full curve) and a kinetically hindered process (dotted curve). Note that the Eo' remains the same for each although the peak to peak separation increase.

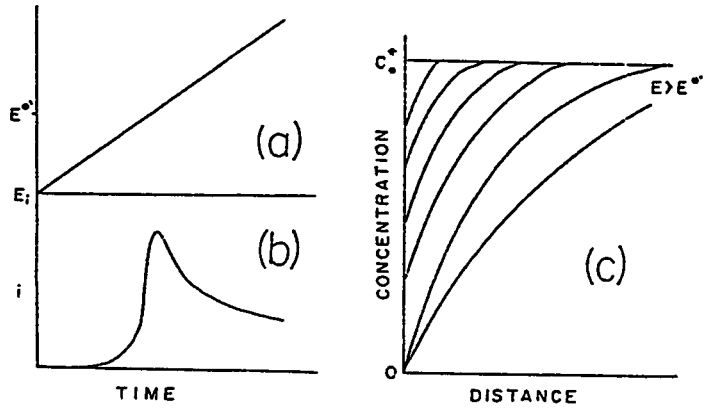


Figure 1.9
(a) Potential scan of linear sweep voltammetry, and
(b) the resulting current response
(c) the concentration profile where the current decays once the concentration of the analyte decreases to zero at the electrode surface.

The current-potential plot obtained on the first forward potential scan of a CV analysis is the same as a LSV voltammogram. The performance of repetitive triangular scans, however, provides more information with fewer experimental restrictions. A complete CV analysis consists of one or more cycles. If there are no coupled chemical reactions, successive cycles show only slight alterations until a steady state is reached. In the presence of coupled reactions, however, notable changes in the plots may occur to reflect those reactions, especially during the first few cycles. The important parameters of a cyclic voltammogram are the magnitudes of the anodic peak current (i_{pa}), the cathodic peak current (i_{pc}), the anodic peak potential (E_{pa}) and the cathodic peak potential (E_{pc}). A redox couple in which both species rapidly exchange electrons with the working electrode is termed as an electrochemically reversible couple. The formal reduction potential ($E^{0'}$) for a reversible couple is centered between E_{pa} and E_{pc}.

$$E^{0'} = \frac{E_{pa} + E_{pc}}{2}$$

The number of electrons transferred in the electrode reaction n for a reversible couple can be determined from the separation between the peak potentials

$$\Delta E_p = E_{pa} - E_{pc} = \frac{0.059}{n} \text{ V}$$

and the values of i_{pa} and i_{pc} should be identical for a simple reversible couple, that is,

$$\frac{i_{pa}}{i_{pc}} = 1.$$

Electrochemical irreversibility is caused by slow electron exchange of the redox species with the working electrode. In this case, the previous equations are not applicable. The irreversibility is characterized by a separation of peak potentials greater than 0.059 / n (14).

The peak current for a reversible system is described by the Randles-Sevik equation for the forward sweep of the first cycle.

$$(i_p)_{rev} = K' n^{3/2} A D^{1/2} C V^{1/2}$$

where K' is constant, A is the electrode area in cm^2, and V is the potential scan rate in V/sec. The peak current for irreversible system is given by the equation,

$$(i_p)_{irr} = K'' n (\alpha n_\alpha)^{1/2} A D^{1/2} V^{1/2} C$$

where α is the charge transfer coefficient and n_α is the number of electrons transferred in the rate determining step.

Both currents $(i_p)_{rev}$ and $(i_p)_{irr}$ can be used for analytical determinations since they are proportional to concentration. However, the peak current for an irreversible system is smaller than that for the reversible system (15).

where ,

$$\frac{(i_p)_{irr}}{(i_p)_{rev}} = 0.77$$

1.4.5 Adsorptive Stripping Voltammetry

Stripping methods are generally based on a preconcentration step before analysis either by forming an amalgam of the analyte and the electrode material or by adsorbing the substance on the electrode surface. Here, a HMDE is used in most cases and immersed in a stirred solution of the analyte for several minutes. Physical adsorption will result in deposition of the analyte on the electrode surface. The strirring is stopped after a sufficient quantity of analyte has accummulated and the deposited material is stripped and determined by linear scan or pulsed voltammetric measurements (Fig. 1.10).

Many organic compounds exhibit surface-active properties that are manifested by their adsorption from solution onto the surface of a solid phase. This phenomenon forms the basis for adsorptive stripping voltammetry (AdSV), where the species to be determined are accumulated on the electrode by adsorption. It follows that the AdSV method should be employed under optimum conditions of base electrolyte, accumulation potential, accumulation time and scan rate, and the resulting electrochemical response signal is proportional to the concentration of analyte at the electrode which in turn proportional to its concentration in the sample solution. When this dependence deviates from linearity, the experimental conditions must be modified (dilution of the solution, decrease of the accumulation time, accumulation under non-stirring conditions). The method of standard additions can be used for the quantitative analysis, and the measurements should be done in thermostatted vessels.

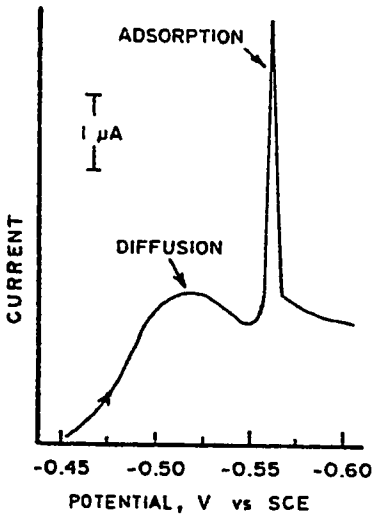


Figure 1.10 Stationary electrode voltammogram at a hanging mercury drop electrode for 0.495 mM Pb^{2+} in 1.0 M NaI, 0.01 M $HClO_4$, scan rate = 0.005 V/s. [From Ref. 15, reprinted with permission from R.W. Murray and D. J. Gros, Anal Chem. 38.392 (1966). Copying 1966 American Chemical Society.

The high sensitivity of adsorptive stripping methods is obviously their greatest advantage. On the other hand, a serious drawback is interference from other surface-active substances that may be present in the solution. In this case, competitive adsorption usually occurs and leads to a decrease in the measured current. Thus, in such cases, it is necessary to separate the interfering compounds.

1.5 Application of Voltammetry to Pharmaceutical Analysis

An extremely large numbers of organic compounds are either directly reducible at the DME or oxidizable at solid electrodes or can be chemically modified to yield electroactive derivatives. No simple rule can be given to predict the voltammetric activity of a certain organic compound in a given potential range. Reactivity depends on the type of bonds broken or formed during electrochemical process, molecular structure, and environment of the bond involved (15). The functional groups which show excellent voltammetric properties include the nitro, nitroso, quinone, azo, azoxy, azromethine, activated carbonyls and activated double bonds (8).

The early successful application of voltammetry to problems of pharmaceutical and biological analysis is best explained by the fact that in drug analysis the samples are well defined, even when they are complicated mixtures of an approximately known composition. The choice of the appropriate technique to solve an analytical-pharmaceutical problem is often controlled by the sample matrix, and the amount of the active

ingredient that is required before the analytical measurement can be made. A dosage form may be dissolved in an aqueous medium, filtered, and analyzed directly by an electrochemical technique or extracted into an organic solvent and analyzed choromatographically or spectrophotometrically.

Perhaps the most important point in this respect is that polarography and voltammetry are among the very few techniques that are equally suitable for analyzing inorganic, organometallic and organic compounds. The good detection limits for drugs offered by differential pulse polarography (dpp) and differential pulse voltammetry (dpv), the commonest techniques in day-to-day analysis, can be further improved by preconcentrating the electroactive species at the electrode by adsorption at the electrode solution interface. Detection limits of 2.5×10^{-11} M were reported for riboflavin (16). The determination of drugs can be performed by differential pulse polarography without any previous separation provided that the drug is more strongly adsorbed on the electrode than the surfactant present in the formulation. In the analysis of drugs in dosage forms, electroanalytical chemistry has been shown to be an exceptional method, and quite often superior to classical wet methods or spectroscopic methods. This may be ascribed mainly to the great ease of sample preparation and lack of interferences from incipients in the dosage form. More recently, fast scan and differential pulse polarography have become extremely useful for measurement of drugs in biological fluids and tissues (17). This can be directly attributed to the introduction of highly sensitive commercial instrumentation which yields easily interpretable data for routine quantification at low levels. In order for polarographic assays to

yield analytically meaningful results for determination of drugs in biological fluids, selective extraction, cleanup, and separation of the parent drug from any metabolites and/or other substances present must be employed prior to voltammetric assay (18, 19). The specificity of the voltammetric methods, usually, excellent because of the compound can be identified by its voltammetric peak or half-wave potential. Examples of voltammetric determination of organic compounds in pharmaceutical preparations include many classes of drugs: tranquilizers, hypnotics, antibiotics, steroids, antihistamines, muscle relaxants, anticoagulants, and others (17). The assay is accurate, sensitive, and since sometimes it does not require the removal of insoluble matter, is more rapid than the spectrophotometric procedures.

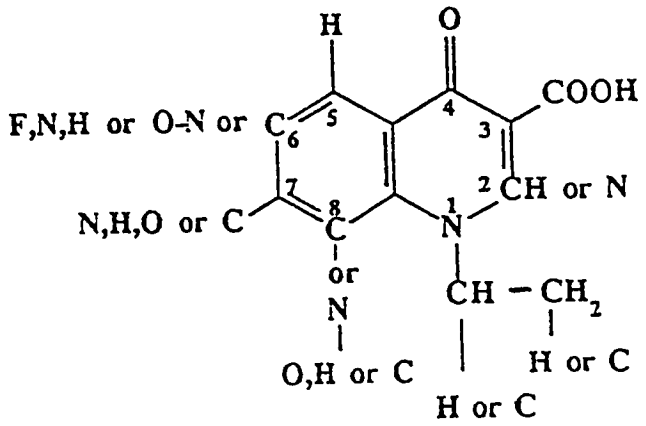
In a development of a drug, analytical methods are required to ensure the purity of starting materials and can be used to monitor a synthetic process to determine if it has reached completion. After synthesis, analysis is required to determine percent yield and to separate, isolate and quantitate by-products of the reaction. Analytical research receives the drug after synthesis and characterizes its physicochemical parameters to ensure that all pharmacological testing is done with pure material and to establish specifications so that future production lots will yield reproducible in vivo response (17). Voltammetric techniques are quite useful for the direct measurement of the stability of some pharmaceuticals in aqueous solution (21, 22). Depending on the compound involved, voltammetry can be used to either monitor the decomposition of a drug and/or the formation of a decomposition product.

Dissolution rate testing is an important aspect of the development of a drug. It can serve as a guide to formulation development and may be correlated to the absorbability potential of some poorly soluble drugs. The silicone-rubber-based graphite electrode has been employed as an appropriate voltammetric sensor to measure the rates of dissolution of several oxidizable pharmaceutical compounds (20, 17). Moreover, the determination of drugs permeability characteristics is done by polarography (17).

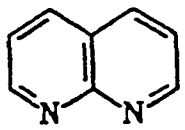
1.6 4-Quinolone Antibacterial Drugs

Antibiotics and antibacterials are compounds used to treat human infections. While both are used to inhibit the life progress of organisms, antibiotics (for example: tetracyclins, penicillins, and cephalosporins) are compounds derived from or produced by living organisms. However, antibacterial drugs are those substances produced synthetically by man to act against bacteria, for example, sulfonamides, nitrofurazone and 4-quinolones.

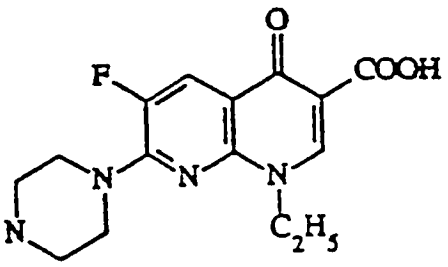
4-Quinolone antibacterial agents are synthetic compounds of a very broad spectrum of antibacterial activity against gram-positive and gram-negative bacteria (32, 33). Infact, the antibacterial potency of norfloxacin (a member of 4-Quinolones group) exceeds that of many other antibiotics and antibacterial drugs. These compounds were classified as 4-Quinolones since they share the common 4-Quinolone skeleton,



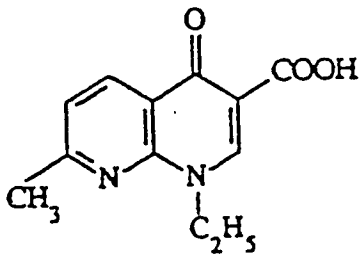
These compounds comprise the following drugs as examples; nalidixic acid and enoxacin as naphthyridine derivatives; norfloxacin, ciprofloxacin, flumequine, acrosoxacin, oxolinic acid, ofloxacin and pefloxacin as quinoline derivatives; cinoxacin as cinolin derivate; pipemidic and piromidic acids as pyrido-pyrimidine derivatives. The structural formulas of these compounds are given below:



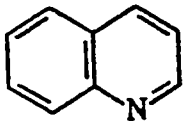
Naphthyridine



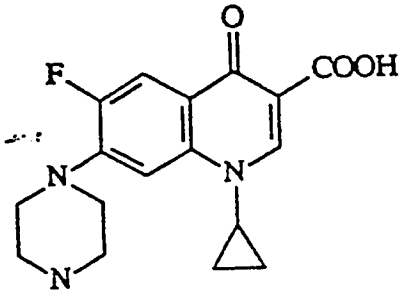
Enoxacin



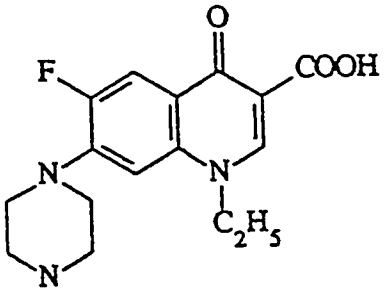
Nalidixic acid



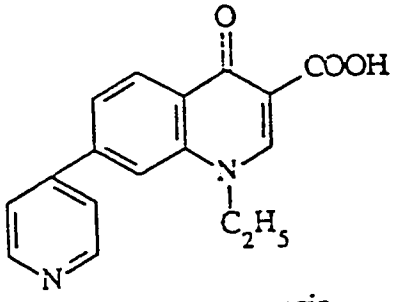
Quinoline



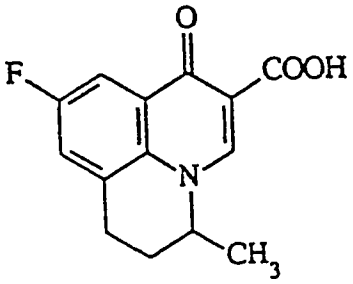
Ciprofloxacin



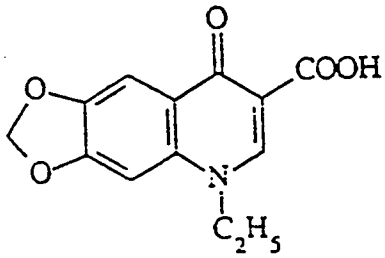
Norfloxacin



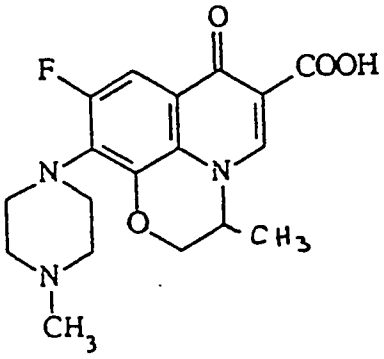
ocrosoxacin



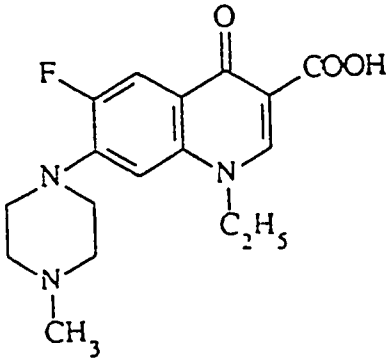
Flumequine



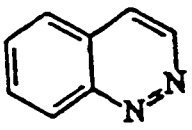
Oxolinic acid



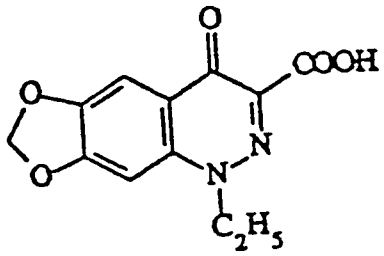
Ofloxacin



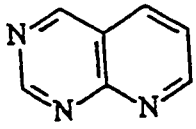
Pefloxacin



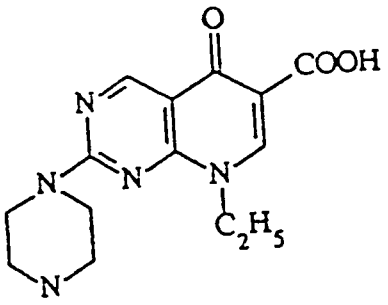
Cinoline



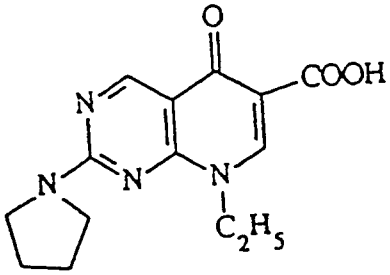
Cinoxacin



Pyrido-pyrimidine



Pipemidic acid



Piromidic acid

1.7 Electroanalytical Assay of 4-Quinolone Drugs

Extensive studies have been reported in the literature regarding the electroanalytical assays of many types of pharmaceuticals (23) and drugs including the antibiotics (23, 24) such as chloramphenicol, tetracyclines, streptomycin, penicillin, cephalosporins, sulfanilamides and others. However, studies concerning voltammetric behavior of 4-Quinolone antibacterial drugs are still limited in the literature. The polarographic behavior of nalidixic acid (the first of this group introduced clinically) was studied (25, 26). It is reported that the protonated and uncharged forms of this acid are reduced at the dropping mercury electrode in two one-electron waves. Meanwhile, its anion is reduced in a single one electron wave. Reduction of nalidixic acid results in hydrogenation of the ethylenic bond in the azinone ring. Nalidixic acid was determined Polarographically in urine (26) (in a 1:1 mixture of methanol and phosphate buffer of pH 4) and in tablets (25) (in 20% dimethylformamide with 0.1 M HCl).

Differential pulse polarographic behavior of Norfloxacin in NaOH has been studied in neutral and slightly alkaline solutions (18). Under these conditions, two reduction peaks occured, at -1.56 and -1.70 V in 0.1 M KCl base electrolyte versus standard calomel electrode. The relationship between peak current and concentration is rectilinear in a wide concentration range. However, the differential pulse polarographic determination of norfloxacin in human serum requires a suitable separation of proteins. The stability of the standard solution of norfloxacin was investigated at ambient environment. It was found that, norfloxacin solution decomposes with time.

A simple and rapid method for the determination of the 4-Quinolone ciprofloxacin by differential pulse polarography has been developed (27). The changes in the voltammetric behavior of this drug with pH was studied, and the most suitable pH for analytical purposes was found to be pH 8.5. The dpp behavior of this compound at this pH exhibited two reduction waves at - 1.44 and - 1.64 V. The former wave could be used to determine ciprofloxacin between 5×10^{-7} and 3×10^{-5} M. The method was applied to the determination of ciprofloxacin in formulated tablets with a relative standard deviation of less than 0.4%. A comparison of adsorptive stripping voltammetry at HDME and carbon paste electrode for the determination of ciprofloxacin in urine was made (19). The antibiotic compound could be preconcentrated at both electrode surfaces, with carbon paste electrodes yielding better performance. These electrodes could be used to determine ciprofloxacin between 6×10^{-7} and 4×10^{-6} M in urine. Ciprofloxacin was separated from the biological matrix prior to adsorptive stripping analysis. The method was applied to the determination of ciprofloxacin in spiked urine samples with a relative standard deviation of 4.1%.

Fluoroquinolone antibacterial agent, ofloxacin, was also studied by adsorptive stripping voltammetry (28). A well defined stripping peak was observed at -1.675 V versus Ag/AgCl reference electrode. Controlled interfacial accumulation of ofloxacin on a static mercury drop electrode in the hanging mercury drop mode provides high sensitivity. The linear concentration range was 0.079 to 197.5 µg/ml. The detection limit of ofloxacin was 1 ng/ml. The precision is excellent with a relative standard

deviation of 0.75%.

Flumequine was found to be reduced at the dropping mercury electrode in Britton-Robinson buffer containing methanol as a solubilizer, over the pH range 5-10 (29). A well defined cathodic wave was produced over this entire range. The wave was characterized as being diffusion controlled, although adsorption pehenomena has a limited role in the reduction process.

The electrochemical behavior of pipemedic acid, a well known antimicrobial agent used for urinary infections, was also investigated by linear-sweep, differential pulse, and square wave voltammetry at a hanging mercury drop electrode (30). Two reduction processes were observed in Britton-Robinson buffers in the acidic media(at -0.569 V and -0.892 V), whereas only one or two processes were obsrved in alkaline solutions. The adsorbed species were measured voltammetrically by using a cathodic process appearing at -0.76 V in 0.1 M $HClO_4$. Linear calibration graphs were obtained in the range 2.5×10^{-9} - 2.0×10^{-7} M. A simple procedure of extraction was employed for the determination of the drug in urine samples. Pipemidic acid was also determined polarographically in capsules in a medium containing 50% acetic acid and dimethylformamide as a solubilizer (31). Britton-Robinson buffer was used as the supporting electrolyte. The reduction was pH dependent with two equal reduction steps at pH 5 and one reduction step at pH 10.

1.8 Objectives

The main objective of this study is to develop adequate, easy, quick and precise electroanalytical procedures for the quantitative determination of some 4-quinolone antibacterial drugs in commercial products and tablet forms. The achievement of such a goal will be the results of the following studies:

1. This study is limited to two respresentative 4-quinolone antibacterial drugs. These include: norfloxacin (quinoline derivative) and nalidixic acid (naphthyridine derivative).

2. Studying the voltammetric behavior of the drugs under variable conditions (various base electrolytes, different pH values, aqueous and mixed solvents) at dropping mercury electrode and hanging mercury drop electrode. Direct current (DC), sampled DC, and differential pulse polarography modes will be used.

3. Investigating the physicochemical properties of the drugs on the DME and the hanging mercury drop electrode using differential pulse, linear sweep cyclic voltammetry, and adsorption stripping voltammetry.

4. Investigating and testing the applicability of the optimum analytical procedures for the drugs assay in the primary commerical products and in formulated tablets.

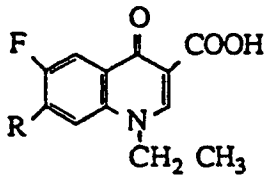
CHAPTER TWO

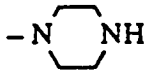
POLAROGRAPHIC BEHAVIOR AND DETERMINATION OF NORFLOXACIN AND NALIDIXIC ACID IN TABLETS

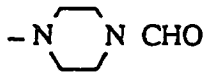
2.1 Introduction:

Norfloxacin (I) is a new fluoroquinolone carboxylic acid (1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7 (1 - piperazinyl) -3- quinoline carboxylic acid) currently in use as a broad spectrum antibiotic (21). It has a potent activity against enterobacteriaceae, i.e. Pseudomonas Spp., Staphylococci and Legionella (18). It is structurally related to nalidixic and oxolinic acids but in terms of its activity against the bacterial agents mentioned above is much more potent (18), and it is also, the main metabolite of pefloxacin, where the hydrogen on the piperazinyl ring of norfloxacin is substituted with a methyl group.

It has been reported that norfloxacin, in its solid state, is stable when stored at 40°C and 75% relative humidity and decomposes when exposed to direct sunlight (21). Two photodecomposition products, formyl piperazine (II) and ethylene diamine (III) analogs have so far, been identified (21).



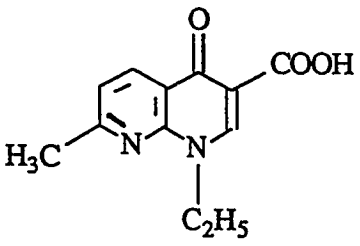
I NORFLOXACIN: R = 

II FORMYL PIPERAZINE: R = 

III ETHYLENE DIAMINE: R = $NH \cdot CH_2CH_2 - NH_2$

Moreover, it is found that, an acidic solution of norfloxacin when heated to 100°C for 15h gave degradation product, namely, the decarboxylate form and no decomposition was observed in alkaline and hydroalcoholic solutions after 15h of exposure at 100°C (21). This phenomenon was ascribed to the acid hydrolysis of norfloxacin, therefore, the work in alkaline solution is recommended (18). Some preliminary studies on the differential pulse polarographic reduction of norfloxacin dissolved in sodium hydroxide have been reported (5).

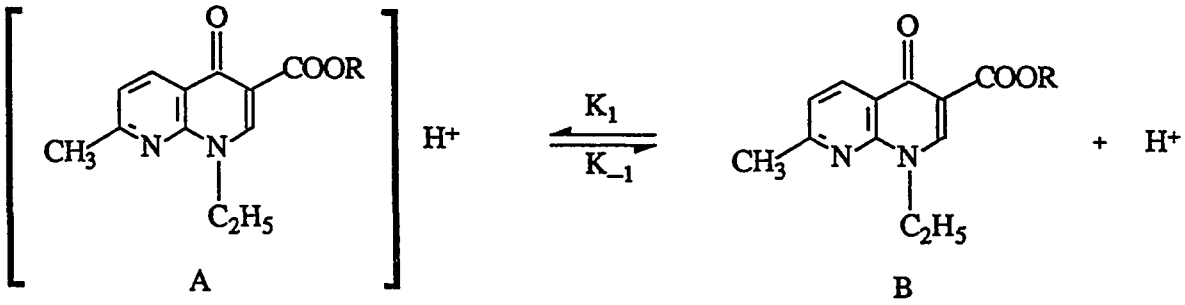
The formula of nalidixic acid (1-ethyl-1,4-dihydro-7-methyl 4-oxo-1,8-naphthyri-dine - 3 - carboxylic acid is:



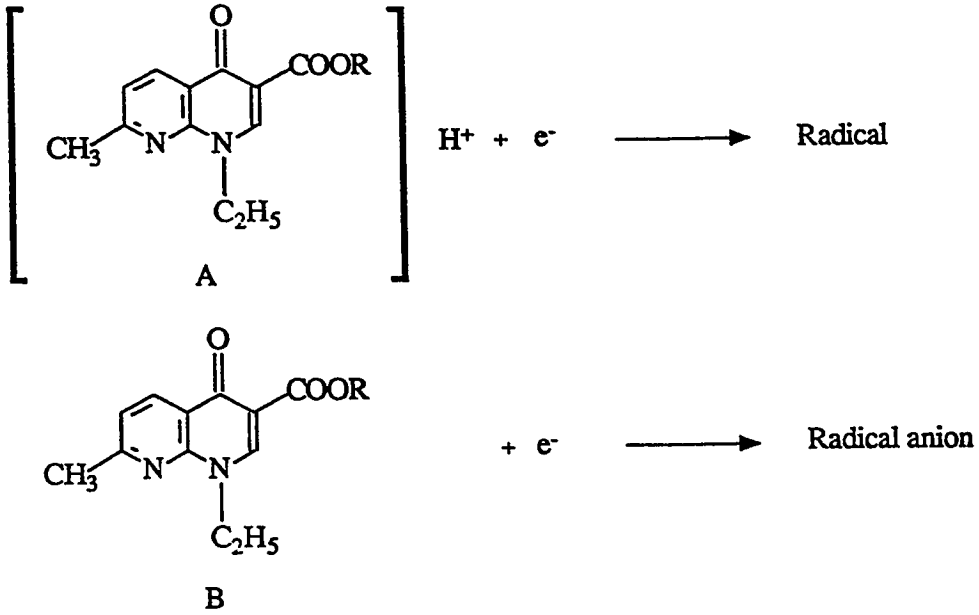
Nalidixic acid

Its propyl ester and its 7-hydroxymethyl derivatives have been the first of the 4 quinolone drug series to be studied by dc polarography in dimethyl formamide medium (26, 32, 33). A mechanism for the reduction of nalidixic acid and its derivatives was proposed. The first compound was propyl nalidixate whose reduction took place in three waves. The first one appeared at a half-wave potential of -0.75V in the strongly acid medium shifting to more negative potential with pH; it became -1.41V at pH about 8.7. The second one was started at -1.55V when the pH was 8.2 and reached -1.62V at pH 12. The third wave as described was a prewave and appeared at $E_{1/2}$ of -1.03V when pH was 5.30 and shifted to -1.20V when pH became 7.40 and disappeared at higher pH values.

It has been suggested, that two forms, protenated (A) and unprotonated (B), exist in the solution with proportions depending on the pH of the medium. Species B predominates at pH > 8.5. However, species A predominates in the acidic medium.



The two waves at about -1.55V (pH 8 to 10) and that at -0.8V to -1.41V (pH 1 to 8.7) were assigned to one-electron step reduction of species A and B respectively.



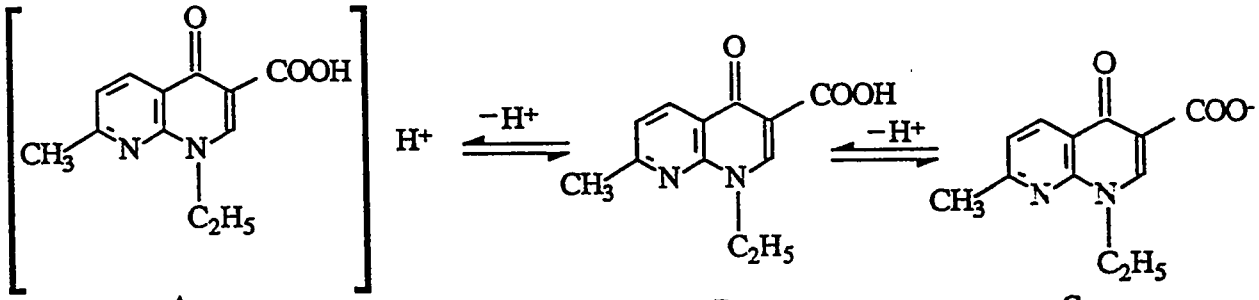
The decrease in the height of the wave starting in the strongly acidic solution by the increase in pH at pH > 7 was attributed to the decrease in the rate of protonation of species B. The wave at -1.55V replaced the other wave at pH > 8.5. The protonation was assumed to be on the pyridine or azinone ring but more likely in the azinone ethylenic bond. The radicals formed in the two reduction steps did not give any indication in the polarogoram. It has been assumed that they may participate in a dimerization, disproportionation or a deactivation reaction with the solvent. The shift in the half wave potential of the wave at -1.55V to more negative potentials at pH ≥ 11 was attributed to the increase in sodium ion concentration in the supporting electrolyte.

The prewave at the most positive potential was attributed to adsorption since it was independent of the concentration of the propyl nalidixate ester at concentration of > 2 x 10^{-4} M.

On the other hand, nalidixic adic (26) showed five waves. The first main wave was observed in the acidic media in the ranges of -0.76V to -1.11V in the pH range of 1 to 6.4. This wave was accompanied with

another one at a more negative potential (- .97V to -1.27V) with nearly same height. The second main wave developed in the pH range of 6 to 10 in the range of -1.08V to -1.23V. This wave was badly developed and accompanied again by another wave at more negative potential (-1.26V to -1.43V) of nearly the same height. The third main wave was developed in alkaline media in the range of -1.58V to -1.68V at pH 9.0 to 13.5.

It has been suggested that nalidix acid exists in solutions of varying pH values according to the following protenation equilibria:



A B C

The first and the third waves appeared in the polaragrams of nalidixic acid were attributed to one electron reduction step of the species A and C which mainly exist in the strongly acidic and alkaline media. This is exactly similar to the case of the propyl nalidixate. The main three waves were attributed to the reduction of A, B and C respectively. The two waves accompanying the first and the second waves were attributed to the reduction of $AH^{\dagger\bullet}$ and $BH^{\dagger\bullet}$ radical species. The following reactions have been suggested for the above mentioned mechanism:

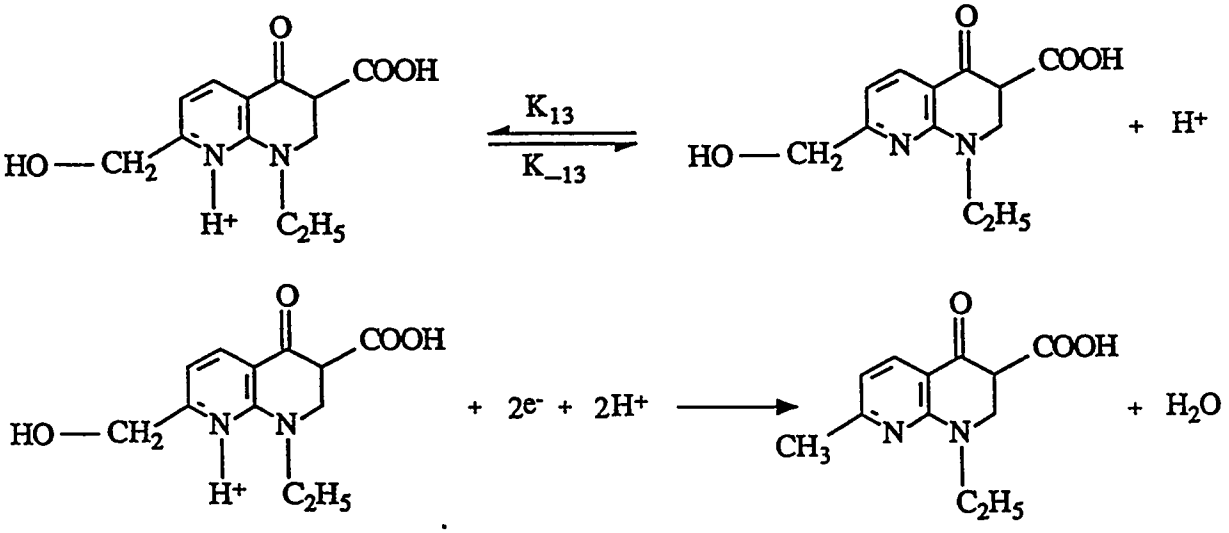
$$A + e^- \longrightarrow A^\bullet$$
$$A^\bullet + H^+ \longrightarrow AH^{\dagger\bullet}$$
$$AH^{\dagger\bullet} + e^- \longrightarrow P_1$$
$$B + e^- \longrightarrow B^\bullet$$
$$B^\bullet + H^+ \longrightarrow BH^{\dagger\bullet}$$

$$BH^{+\bullet} + e^- \quad \text{----->} \quad P_2$$

$$C + e^- \quad \text{----->} \quad C^{\bullet}$$

where P_1 and P_2 are the final products. The waves associated to the reduction of species B to a radical ($B^{\bullet}$) and to the reduction of the protonated radical ($AH^{+\bullet}$) to give the product (P_1) in the case of nalidixic acid did not appear in the case of propyl nalidixate which has been attributed to the the replacement of the grouping COOH by COOR and to the fast deactivation of the radical $A^{\bullet}$ by dimerization, disproportionation or reaction with the solvent or by a shift of $E_{1/2}$ to potentials more negative than those of the final rise of the current respectively.

The 7-hydroxymethylnalidixic acid showed a pattern similar to nalidixic acid where waves corresponding to the protonated, neutral and unprotonated species were observed exactly similar to nalixidic acid. A new wave appeared in strongly acidic medium which was ascribed to the reduction of the C-O bond in the side chain in the protonated form as follows:



The polarographic behavior of norfloxacin and nalidixic acid in the presence of dimethyl formamide has been further examined here using dc and dpp in more details and applied for their determination in commercial tablets.

2.2 Experimental Procedures and Methods

2.2.1 Chemicals and Equipment

Norfloxacin was supplied by SIEGFRIED AG/LTD, Switzerland, nalidixic acid from Fluka and all supporting electrolytic salts were of analytical reagent grade. Tablets of norfloxacin (Noroxin) and nalidixic acid (Negram) were obtained from Merck Sharp and Dhome, Netherlands and Winthrop Laboratories, England respectively. Purum grade dimethyl formamide (DMF) was from Fluka. Distilled deionized water was used in preparing all solutions.

A PAR 174 (EG&C) polarographic analyzer in conjunction with the static mercury drop electrode Model 303 with a small size drop and X-Y recorder (Model RE 0074) were utilized. A cell of three electrode system (DME as the working electrodes, Ag/AgCl as the reference electrode and platinum auxiliary electrode) was used. Sample solutions were deaerated with oxygen free nitrogen and the temperature was 25 $\pm 1^{\circ}C$. A differential pulse amplitude of 25 mV and a scan rate of 5 or 10 mV/s were used. EG&G Model 264A Polarographic Analyzer/Stripping Voltammeter in conjunction with X-Y recorder (Model RE 0150) have been used to record

the cyclic voltammograms. A pH meter (Corning 215) was used for measuring and adjusting the pH of the base electrolytes.

2.2.2 Analytical Procedure

10.0 ml of 0.1 M of the base electrolyte was transferred to the polarographic cell and deaerated for eight minutes. A stock solution of 0.01 M norfloxacin or nalidixic acid prepared in dimethyl formamide was spiked into the base electrolyte. The dc or differential pulse polarographic current voltage curves were recorded after each addition. The limiting currents were measured and calibration curves in several base electrolytes were constructed.

2.2.3 Sample Preparation

Eight tablets of Noroxin or Negram were ground to a fine powder. A quantity equivalent to one tablet was weighed, dissolved in DMF, transferred into a 100 ml volumetric flask and diluted to the mark with DMF. The solution was slightly turbid but no further treatment was required. Known volumes (0.05 to 0.5 ml) of the sample solution were added to 10 ml aliquots of the base electrolyte. The percent composition of DMF was kept constant at about 6% in the unknown and standard solutions. The total volume of the sample was kept as 10.60 ml. Calibration solutions were prepared in the same manner.

2.3 Results and Discussion

2.3.1 Reversibility of the main electrochemical reactions for Norfloxacin

It has been mentioned in Chapter 1 that the reversibility of a dc electrode process could be assessed by the Hyrovsky - Ilkovic equation,

$$E = E_{1/2} - \frac{0.0591}{n} \log \frac{i}{i_d - i}$$

at 25°C. The graphical plot of E vs. log $i/(i_d - i)$, should be linear with a slope of 59.1/n mV. An alternative criterion for the reversibility is to measure the difference $| E_{1/4} - E_{3/4} |$ from the polarogram where $E_{1/4}$ and $E_{3/4}$ correspond to values of E at (1/4) i_d and (3/4) i_d respectively. The difference was suggested to be 56.4 mV at 25°C (3). For the irreversible processes, the Hyrovsky-Ilkovic equation was developed and given in this form:

$$E = E_{1/2} - \frac{0.0591}{\alpha n_a} \log \frac{i}{i_d - i}$$

where α is the charge transfer coefficient and n_a is the number of electrons transferred in the rate determining step. αn_a is a value ranging from 0 to 1. Similarly $E_{1/4} - E_{3/4}$ are larger than those for the reversible case. $E_{1/2}$ is also a function of drop time and the rate constant of the electrochemical process. Thus, wave shape, wave position and drop time dependance of $E_{1/2}$ are criteria for the reversibility in the case of dc polarography.

In the case of dp polarography, the dp peak half width, $W_{1/2}$ was suggested to be 90.4/n mV at 25°C for a reversible process (3). It has been reported (34) that reversibility could be diagnosted on the basis of recording the dp polarogram with a negative going potential pulse and then repeating the dp polarogram with a positive potential pulse. Thus, for a reversible system,

$$E_p^c - E_p^a = |\Delta E|$$

where E_p^c and E_p^a are the cathodic and anodic peak potentials respectively, ΔE is the pulse amplitude. Also, $i_p^a / i_p^c = 1$ for a reversible system. where i_p^a and i_p^c are the anodic and cathodic currents.

For an irreversible system,

$$E_p^c - E_p^a \approx |\Delta E|$$

and, $i_p^a / i_p^c < 1$

Various buffered and unbuffered electrolytic solutions of 0.1 M concentrations have been utilized as base electrolytes. The direct current (dc) and differential pulse (dp) polarographic data for 1×10^{-4} M norfloxacin in 1% DMF are reported in Table 2.1. Two main well defined waves in the ranges of -1.42V to -1.58V (wave C) and -1.74V to -1.85V (wave D) have been obtained in each of the base electrolytes used (Fig. 2.1, polarogram 1). Table 1 shows also values for αn_a obtained from the analysis of the log $(i_d - i) / i$ from the dc polarograms and $W_{1/2}$ (the dp peak half width) from the dp polarogram for the two main waves C and D.

Table 2.1 : Polarographic data for norfloxacin in various base electrolytes.

Base Electrolyte/	Wave C				Wave D			
	$E_{1/2}$ (V)	$E_{3/4} - E_{1/4}$ (mV)	αn_a	E_p (V)	$W_{1/2}$ (mV)	$E_{1/2}$ (V)	$E_{3/4} - E_{1/4}$ (mV)	αn_a	E_p (V)	$W_{1/2}$ (mV)
LiCl	-1.55	92	0.49	-1.52	75	-1.85	54	0.73	-1.84	93
KCl	-1.53	85	0.54	-1.48	130	-1.78	35	0.98	-1.79	67
$LiClO_4$	-1.56	93	0.53	-1.55	153	-1.84	52	0.67	-1.85	93
NaOAc/HOAc (pH = 7.4)	-1.56	50	0.64	-1.54	82	-1.88	58	0.36	-1.87	120
NaOAc	-1.59	105	0.43	-1.55	130	-1.88	52	0.54	-1.86	80
K_3 Citrate	-1.53	55	0.84	-1.53	93	-1.81	45	0.70	-1.93	71
K_3 Citrate/K_2H.Cit (pH = 8.0)	-1.57	65	0.59	-1.55	40	-1.89	60	0.40	-1.87	95
NH_3/NH_4Cl (pH = 9.0)	-1.68	65	0.72	-1.67	100	-1.88	40	0.59	-1.85	67
K_2HPO_4	-1.55	75	0.56	-1.59	93	-1.79	110	0.64	-1.80	60
$K_2B_4O_7$	-1.57	75	0.61	-1.61	117	-1.86	120	0.52	-1.79	93

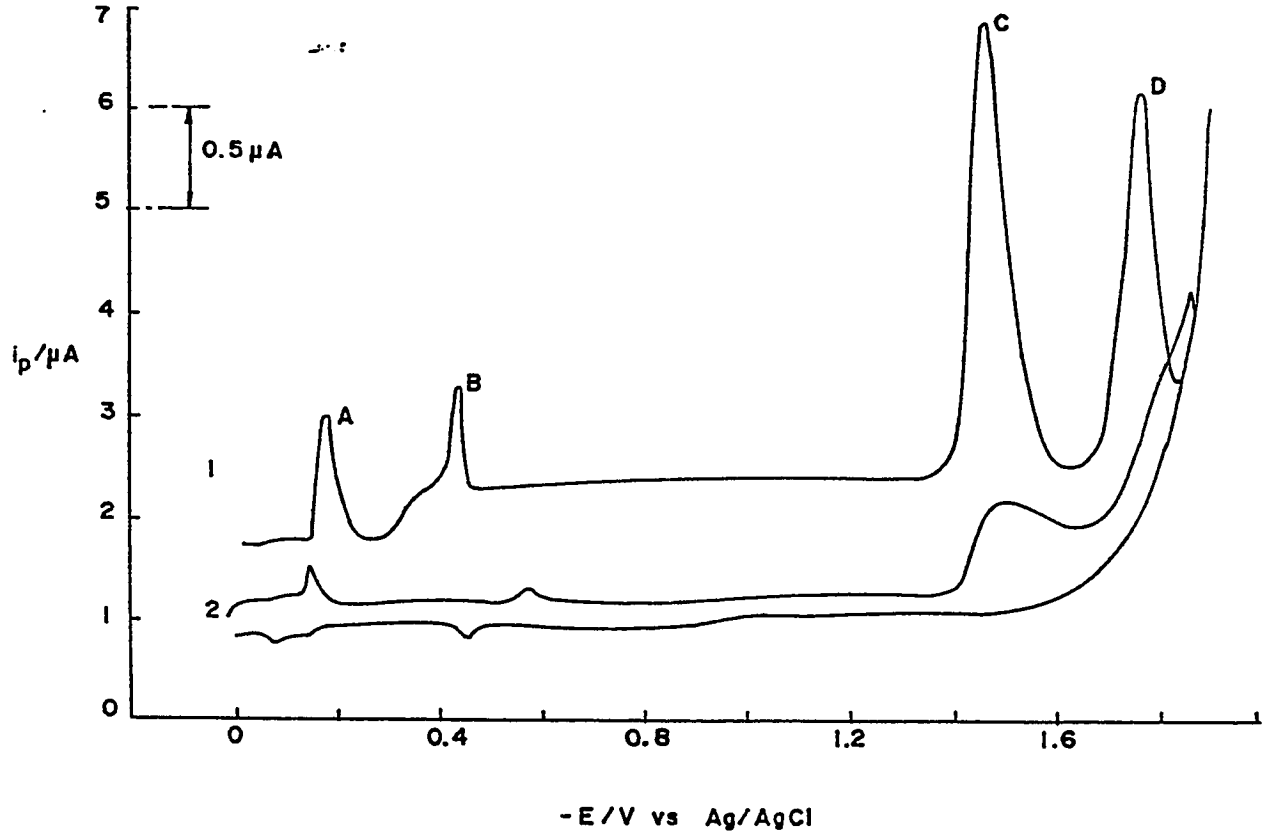


Fig. 2.1 Dp polarogram(1) and cyclic voltammogram(2) for norfloxacin in 0.1 M sodium acetate in the presence of $1X10^{-4}$ M norfloxacin and 0.1% DMF. Scan rate was 10 mV/s for the dp polarogram and 100 mV/s for the cyclic voltammogram.

In general, αn_a and $E_{3/4} - E_{1/4}$ values obtained from the analysis of the dc waves (maximum currents) and $W_{1/2}$ from the dp waves are far from those given for the reversible waves (3). Thus, the rate determining step is irreversible for the two waves. This has also been confirmed from the preliminary runs of cyclic voltammograms for norfloxacin in various base electrolytes (Fig. 2.1, voltammogram 2). The waves corresponding to the dp peaks C and D on the cyclic voltammogram did not appear in the anodic scan. This confirms the irreversibility of the two waves.

2.3.2 Adsorption Waves associating the main electrode processes for Norfloxacin

Two ill defined waves (Fig. 2.1 polarogram 1, waves A and B) were observed at a potential more positive than the main electrochemical waves. The dp peaks of these two waves started to appear with base electrolytes of pH $\geq$ 6.5 and norfloxacin concentration of 5×10^{-5} M at -0.06V and -0.20V. Waves A and B showed an increase in height and anodic shift in potential with norfloxacin concentrations up to 2×10^{-4} M after which they became almost concentration independent. The two waves were significantly affected by DMF. Fig. 2.2a shows that an increase in DMF ratio in the sample solution is associated with a cathodic shift for wave A and anodic shift for wave B with both decreasing in height until they disappeared completely when the DMF ratio exceeded 7%. These observations may indicate that the two waves are of adsorptive nature belonging to the reduction of norfloxacin species available in the solution at that pH range.

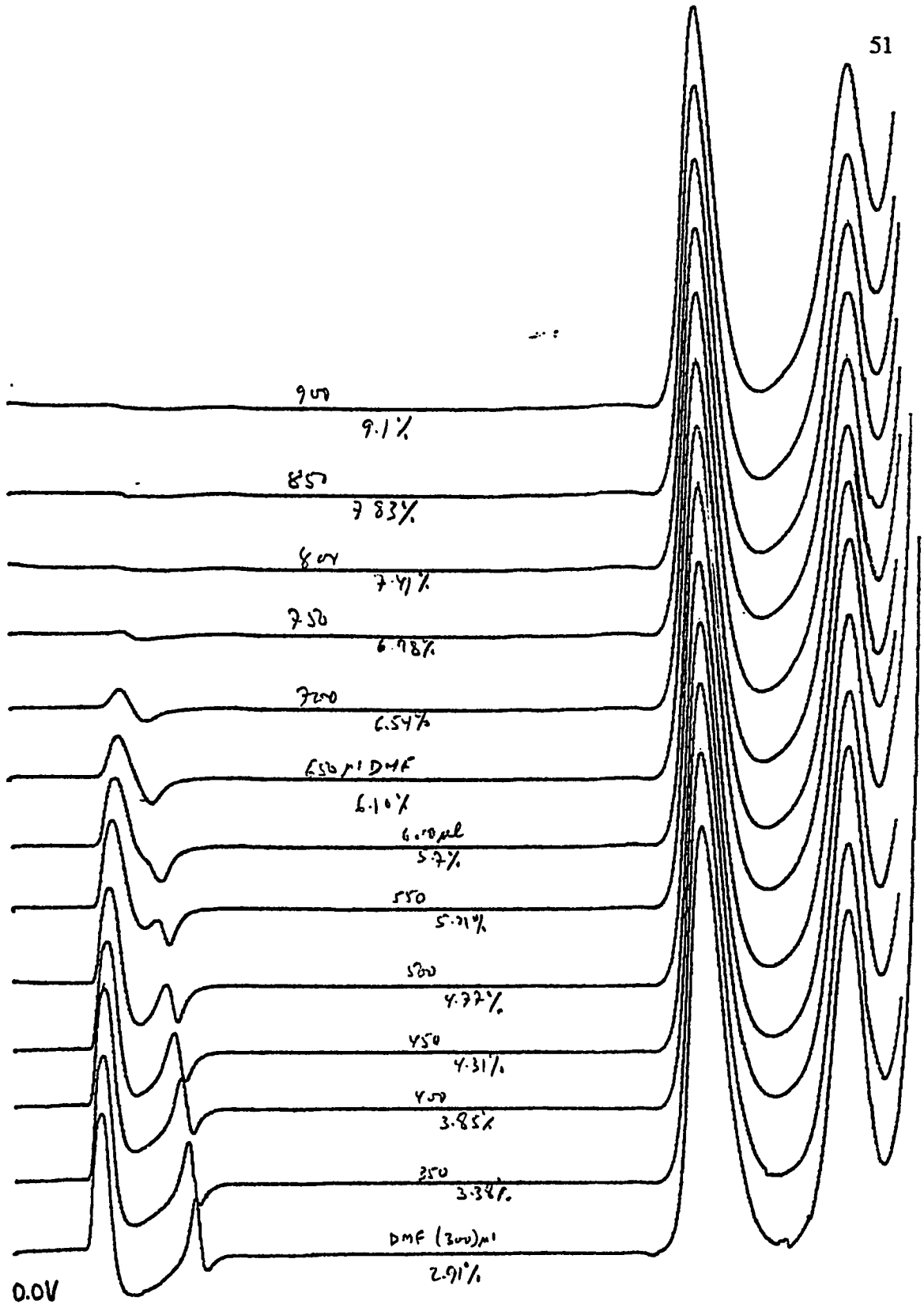
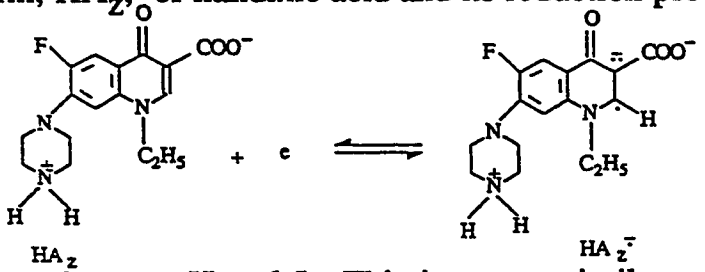


Figure 2.2a. Effect of DMF on the two small adsorptive peaks to the most positive potential. Scan rae used was 10 mV/sec and current sensitivity was 5μA.

The two waves A and B may be ascribed to the adsorption of Zwitterion form, $HA_{Z'}$ of nalidixic acid and its reduction product $HA_Z^{\cdot-}$ at



HA_Z $HA_Z^{\cdot-}$

the electrode surface at pH > 6.5. This is a case similar to the quinone-semiquinone-quinol system which showed two adsorption prewaves which were attributed to quinone and semiquinone (10).

2.3.3 Effect of Dimethyl Fermamide on the Main Electrochemical Waves

Fig. 2.2b shows also that the peak heights and peak potentials of waves C and D are almost independent of DMF ratio in the sample solution up to about 6% above which some insignificant changes were observed. pH was measured after each addition and found almost constant (about 7.8) until the DMF ratio of about 6.5% above which it started a gradual increase (Table 2.2). An increase in the organic solvent content results in an increase of the pH and consequently an increase in the dissociation constant of the protonated species (10). These two effects may lower the rate of protonation and consequently lead to a shift in Ep towards a more negative potential where protonation precedes electron transfer. The shrinkage observed in the heights of waves C and D (Fig. 2) may be due to an increase in the viscosity of the medium, ion-pair formation, and a decrease in adsorbability on the electrode surface due to the increase in the organic solvent. These effects may result in retardation of the electrode process (35).

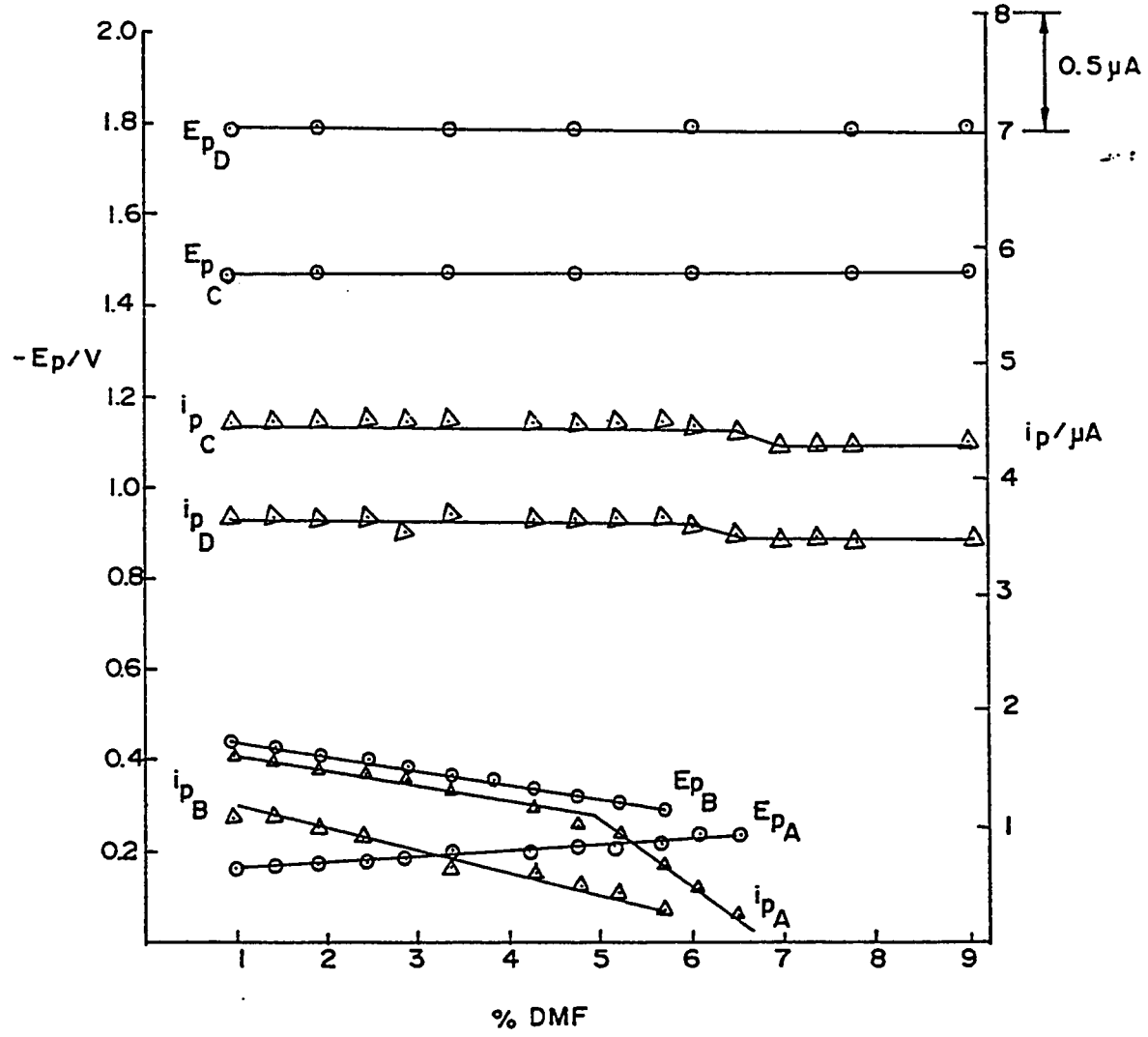


Fig 2.2b The effect of DMF on the dp polarographic peak height and peak potential for $1X10^{-4}$ M norfloxacin in 0.1M sodium acetate. E_p and i_p represent the peak potential and the peak height respectively.

Table 2.2: Effect of norfloxacin and DMF concentrations on the pH of different base electrolytes used. Volume of the 0.1M base electrolyte was 10 ml, concentration of norfloxacin was 0.01M in DMF.

Base Electrolyte K_3 Citrate		Base Electrolyte $K_2B_4O_7.4H_20$		Base Electrolyte K_2HPO_4	
Volume (ml)	pH	Volume (ml)	pH	Volume (ml)	pH
0	8.500	0	9.375	0	9.150
0.02	8.500	0.05	9.375	0.005	9.125
0.03	8.450	0.10	9.375	0.010	9.100
0.10	8.450	0.15	9.375	0.020	9.100
0.15	8.425	0.20	9.400	0.040	9.075
0.20	8.400	0.25	9.425	0.080	9.050
0.25	8.375	0.30	9.450	0.10	9.025
0.30	8.375	0.40	9.475	0.20	9.000
0.35	8.350	0.50	9.500	0.30	8.975
0.50	8.350	0.60	9.525	0.40	8.975
0.60	8.325	0.70	9.550	0.50	8.975
0.70	8.300	0.80	9.575	0.70	8.950
1.30	8.300	0.90	9.625	0.90	8.950
1.40	8.275	1.00	9.650	1.10	8.925
2.00	8.275	1.10	9.675	1.50	8.925

2.3.4 pH Effect on the Polarographic Behavior of Norfloxacin

Figs. 2.3 and 2.4 show dp polarograms for 1 x 10^{-4} M norfloxacin in the presence of 0.1% DMF and 0.1 M of various base electrolytes, namely, hydrochloric acid solutions (Fig. 2.3), acetic acid/sodium acetate buffers, un-buffered sodium acetate and ammonia/ammonium chloride buffers (Fig. 2.4). Table 2.2 shows the buffering capability of some electrolytic salts used as base electrolytes in the presence of DMF and norfloxacin. It is obvious the pH does not change significantly.

It has been observed that norfloxacin shows only one reduction wave at a potential in the range of -0.95V to -1.05V in 4M, 2M and 0.1M HCl base electrolyte. The wave has diminished in height and became a shoulder in 0.1M HCl and finally disappeared in a solution of 0.01M HCl (Fig. 2.3). When pH has been raised to higher values, the above mentioned reduction wave has been replaced by main and ill defined waves in the range of -0.06V up to -1.85V. At pH 6.5, only one main wave appeared at -1.39V and one ill defined wave at -0.11V (Fig. 2.4, polarogram 2). However, when pH of the solution became in the range of 7.5 to 10, two main reduction waves in the ranges of -1.42V to -1.58V (wave C) and -1.74V to -1.85V (wave D) and two ill defined waves in the ranges of -0.11V to -0.18V and -0.20V to -0.42V developed completely (Fig. 2.4 polarogram 3). The two ill defined waves have been disappeared in ammonia/ammonium chloride buffers of pH > 9. They were obscured by a huge wave situated around 0V. Finally only one reduction wave in the range of -1.74V to -1.85V remained when the pH of the base electrolyte

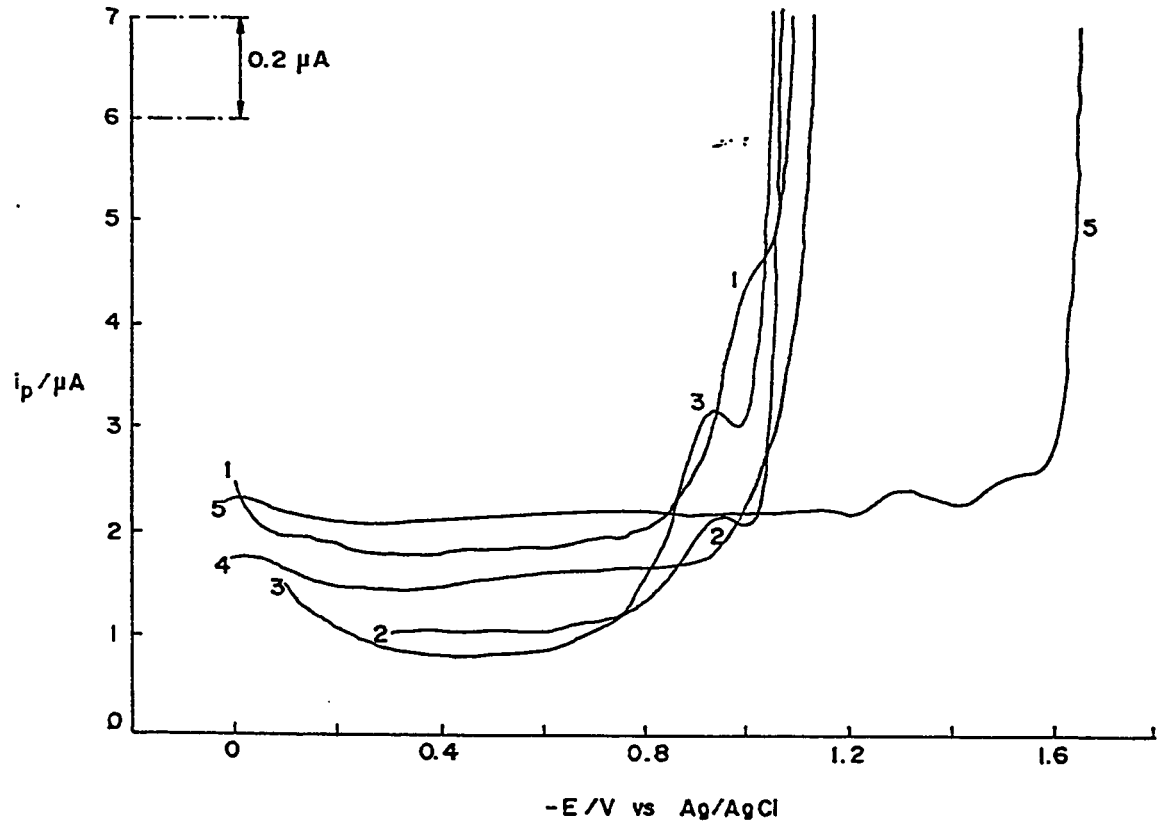


Fig. 2.3 Dp polarograms for $1X10^{-4}$ M norfloxacin in hydrochloric acid solutions of various concentrations in the presence of 0.1% DMF. Polarograms 1,2,3 and 4 belong to 0.1M, 4M, 2M and 0.01M HCl base electrolyte solutions. Polarogram 5 belongs to 0.1 M NaOH base electrolyte.

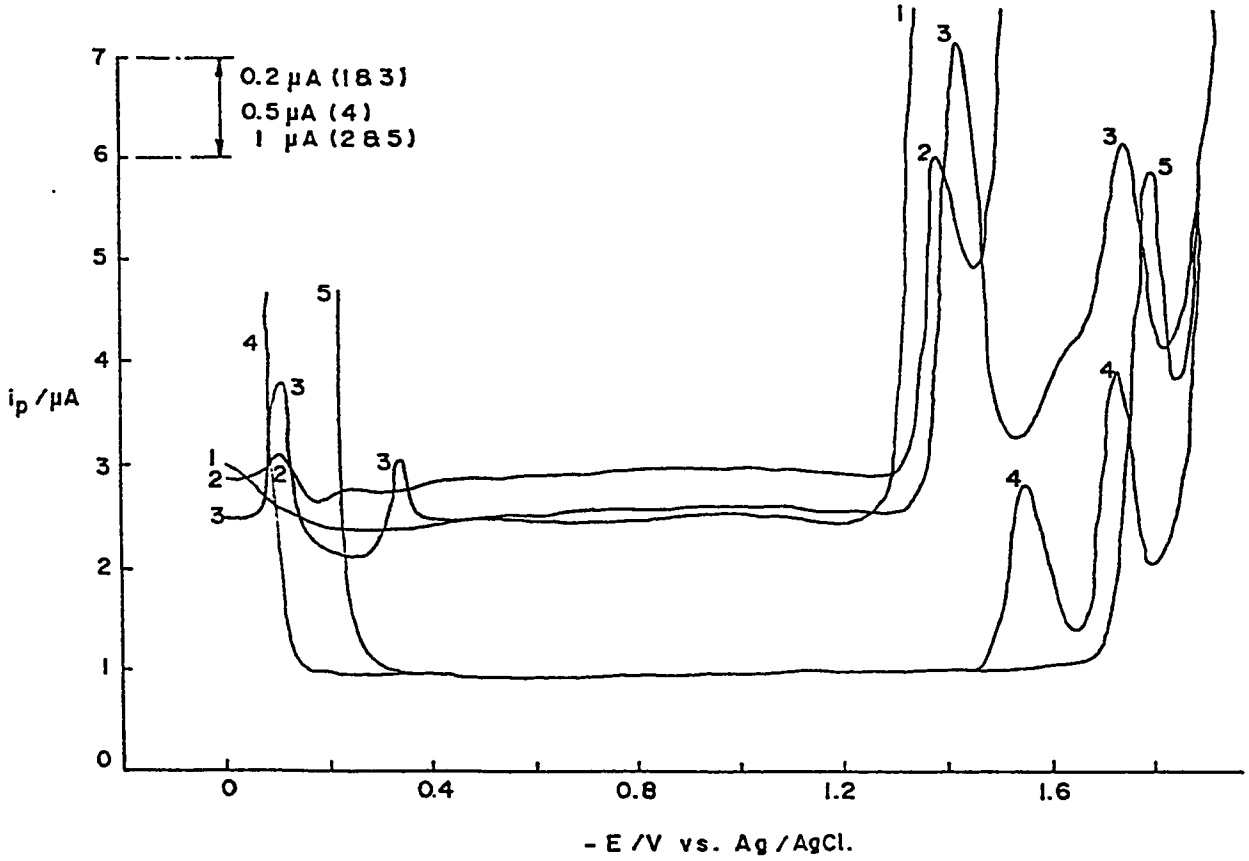


Fig. 2.4 Dp Polarograms for $1X10^{-4}$ M norfloxacin in HOAc/NaOAc buffers (pH in the case of polarograms 1,2 and 3 was 4.5, 6.5 and 7.5 respectively) and in NH_3/NH_4Cl buffers (pH in the case of polarograms 4 and 5 was 9.0 and 11.5 respectively) in the presence of 0.1% DMF.

became in the range of 10 to 11.5. When 0.1M sodium hydroxide was used as the base electrolyte, all waves vanished completely (Fig. 2.3, polarogram 5).

Fig. 2.5 shows the shift in the dp polarographic peak potentials for the four peaks with pH. Ep - pH dependence for wave C is described by two segments of E_p/pH of 39.8 mV and 140 mV below and above pH 8.5 respectively (Fig. 2.5). E_p/pH for wave D was found to be -14mV and 36 mV below and above pH 9.5 respectively (Fig. 5). Other members of anti-bacterial drugs of the same type, namely, nalidixic acid (26), ciprofloxacin (27) and flumequine (29) showed a similar behaviour. The shift of E_p with pH towards more negative potential may indicate that the electron uptake is preceded by a proton transfer as proposed earlier for nalidixic acid. Previously (6), plots of $E_{1/2}$ vs. pH for the polarographic reduction of many organic compounds showed several linear segments indicating different protonation equilibria, the slope of each is proportional to the total number of protons consumed in the reduction process. Thus, the neutral, HA, and the anionic, A^-, forms of norfloxacin may be reducible at the electrode in the intermediate pH ranges. Such a behaviour will be associated by two reduction waves indicating that the recombination of H^+ and A^- in the electrode vicinity takes place at a finite rate (10).

Fig. 2.6 shows the change in the peak heights of the two waves C and D with pH. The plot representing the dp peak height of wave C versus pH shows a break at pH 8. This pH may correspond to the polarographic pK (10) for norfloxacin which may be shifted from the thermodynamic pK of

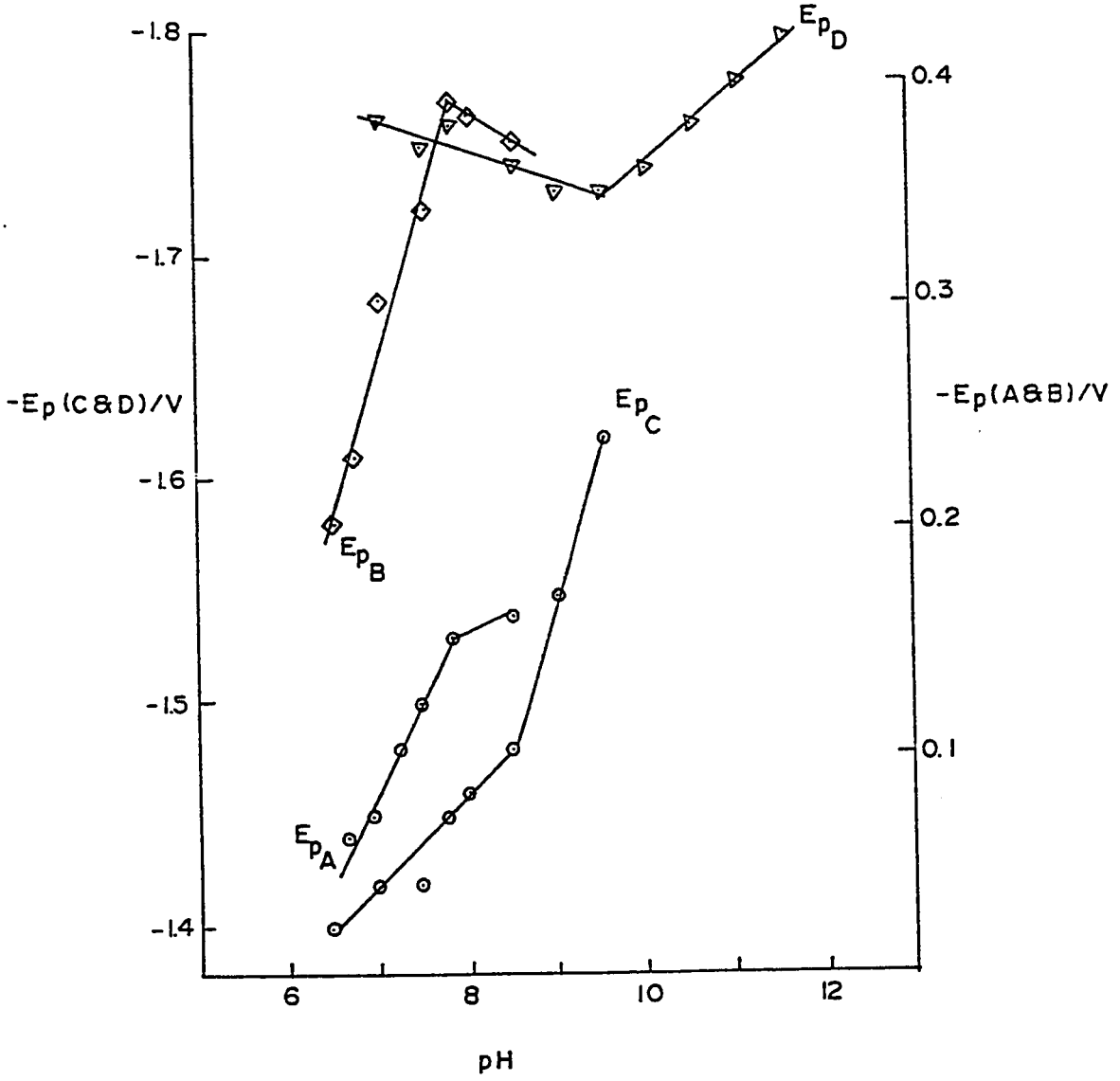


Fig. 2.5 Dp peak potentials vs. pH for $1X10^{-4}$ M norfloxacin in various buffering systems made of HOAc/NaOAc and NH_3/NH_4Cl covering pH range of 4.5 - 11.5.

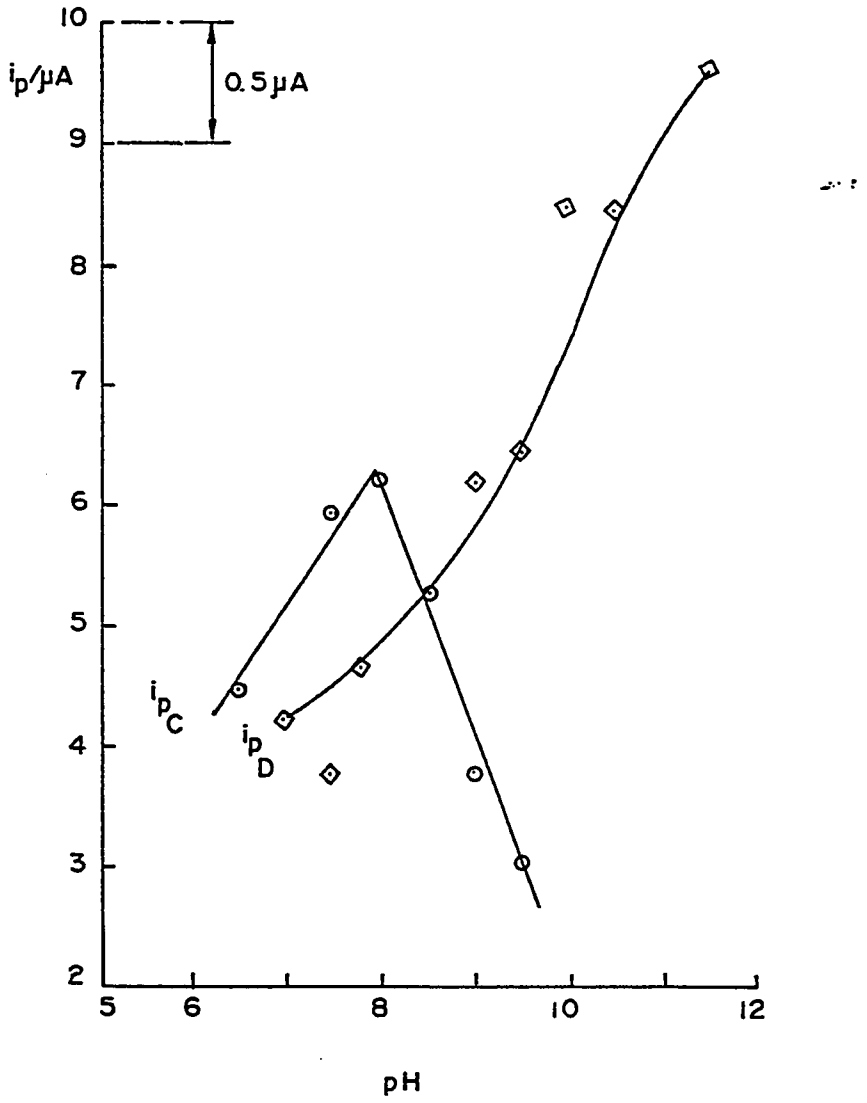


Fig. 2.6 Dp peak heights vs. pH for $1X10^{-4}$ M norfloxacin in various buffering systems made of HOAc/NaOAc and NH_3/NH_4Cl covering pH range of 4.5 - 11.5.

the carboxylic group as was suggested for nalidixic acid (Fig. 2.7). This behaviour has also been observed previously for ciprofloxacin (27) which showed a dp polarographic wave at -1.44V whose height was markedly dependent on pH reaching a maximum at pH 8.5 which was assumed to be the pK value for ciprofloxacin. It has been suggested in the case of nalidixic acid that the wave corresponding to this dp peak (wave C here) belongs to the reduction of the ethylenic bond in the azinone ring. At pH > pK, the proton transfer is slow and the process appears to be kinetically controlled.

2.3.5 Proposed Mechanism for Norfloxacin Reduction at the DME

Norfloxacin is expected to exist in aqueous media with intermediate pH range (≈ 7) as a Zwitterion, HA_Z, a neutral molecule, HA_N and a small proportion of the conjugate acid H_2A^+. It is expected that Zwitterion, HA_Z, will be the major species since one of the nitrogens in the piperazine moiety is more basic than the carboxylate ion. However, in a basic medium (pH $\geq$ 10) the conjugate base A^- predominates. Meanwhile, in a strongly acidic medium (2M HCl, for example) the diprotonated species, H_3A^{2+} would be the major one existing in the solution. The enamine double bond in the azinone ring is protonated in a strongly acidic medium leading to the formation of the iminium ion moiety as in H_3A^{2+}. The above mentioned equilibria are indicated in Scheme I below. As the base electrolyte concentration decreases to $\leq$ 0.01 M HCl, the concentration of H_3A^{2+} species is expected to decrease drastically which is indicated by the disappearance of the dp polarographic wave at -1.02V.

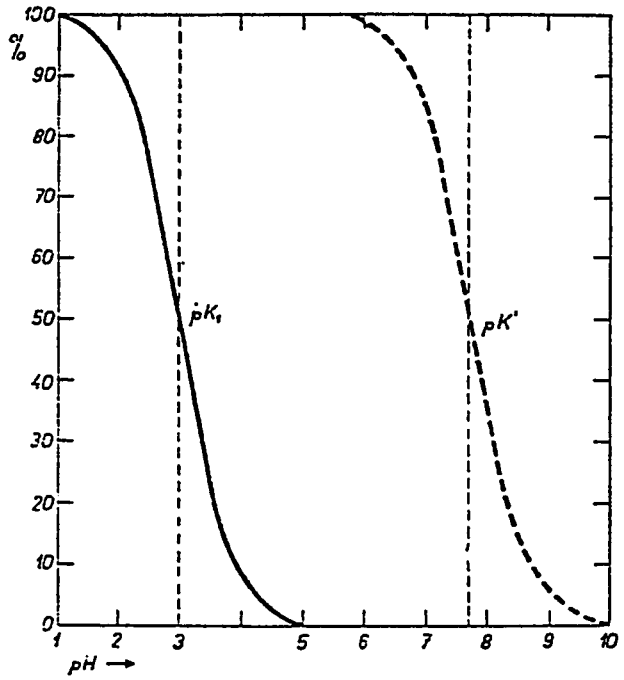
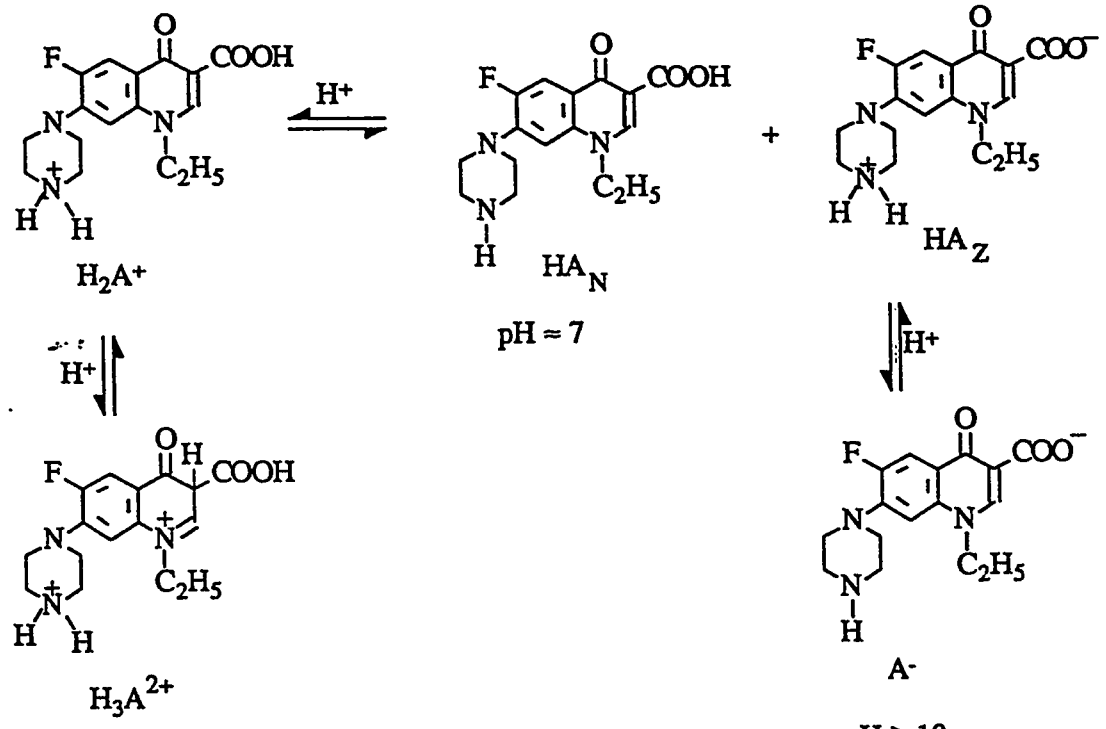


Fig. 2.7 Comparison of titration (full line) and polarographic (dotted line) dissociation curves. The shift is due to the recombination reaction.

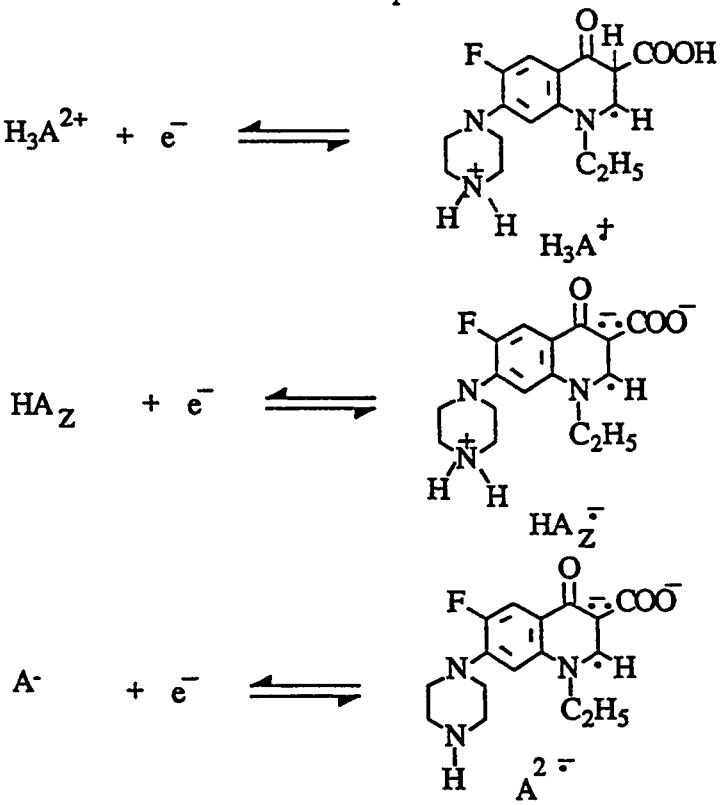
Based on the dp polarograms in base electrolytes of wide pH range, appearance and disappearance of waves according to pH values of the solutions and the above mentioned proposed equilibria, the reduction of norfloxacin at the DME may take place according to the equations of Scheme II.

Thus, the wave at -1.02V in strongly acidic media of HCl solutions and waves C and D in the intermediate and basic media could be attributed to one-electron reduction steps of the species, H_3A^{2+}, HA_Z and A^- respectively. This reduction will produce the radical products: $H_3A^{\ddagger}$, $HA_Z^{\bullet}$, and $A^{2\bullet}$ as indicated in Scheme II.

The radicals produced as a result of electrochemical reduction, at the DME may deactivate by dimerization, disproportionation or reaction with the solvent as suggested for nalidixic acid (26). It may also be suggested that the radicals may undergo further one-electron reduction step producing waves shifted to potentials more negative than those of the final rise of the currents. The absence of waves related to the species, HA_N and H_2A^+ may be ascribed to their limited proportions in solutions at an intermediate pH range.



Scheme I: Acid-base equilibria for norfloxacin in aqueous media.



Scheme II: Electrochemical reduction of norfloxacin at the DME.

2.3.6 Linearity of Calibration Plots for Norfloxacin in Various Base Electrolytes

The concentration dependence of the dp polarographic peak heights of norfloxacin in the presence of various base electrolytes was checked for the two well defined peaks C and D at pH values of slightly acidic to slightly alkaline solutions. The linearity was tested for norfloxacin concentration in the range of 2 to 560 ppm (5×10^{-6} to 1.7×10^{-3} M). The results are shown in Table 2.3 and Fig. 2.8. At low concentrations, more than one linear segment have been observed. A curvature has also been observed in the calibration curves in some cases. The breaks or curvature in the calibration curves may be ascribed to a change in the mechanism of reduction at the electrode which is dependant upon the protonated, uncharged, and anionic species of norfloxacin. It has been observed also that the dp peak potentials are concentration dependent. Peak C showed a significant shift to a more positive potential at concentrations less than about 5×10^{-5} M above which the direction of the shift has been reversed to the opposite direction i.e., to a more negative potential. This behaviour may be attributed to the presence of the anionic species of norfloxacin at low concentrations rather than the undissociated forms. The shift in the potential of peak D with concentration was in the negative potential direction.

The dp peak C was always a well defined wave and far from the discharge of the base electrolyte with a reasonable linearity within a rectilinear range of about 32 to > 560 ppm (9.9×10^{-5} to > 1.7×10^{-3} M) norfloxacin. Thus, this peak was used to test the validity of the method for determination of the drug in commercial tablets.

Table 2.3: Linearity of calibration plots for norfloxacin in 0.1M solutions of various base electrolytes.

	Wave C			Wave D		
Base Elect.	Range ppm	Corr. Coeff.	Slope ($X10^{-2}$) $\mu A.L.mg^{-1}$	Range ppm	Corr. Coeff.	Slope ($X10^{-2}$) $\mu A.L.mg^{-1}$
$K_2B_4O_7$	0-94	0.9990	2.836	16-94	0.9983	2.93
	94-320	0.9994	2.189	94-320	0.9995	2.108
K_3 Citrate	0-16	0.9995	5.436	0-10	0.9980	6.00
	16-35	0.9999	3.781	10-35	0.9970	3.228
	6-540	0.9986	1.259	continuous curvature with new peak starting at -1.94 V		
LiCl	0-20	curved	---	0.25	0.9983	5.120
	16-400	0.9993	1.141	124-345	0.9987	1.361
KCl	0-10	0.9976	6.988	2-13	0.9996	5.455
	13-30	0.9996	3.571	2-13	curved	
	32-560	0.9997	1.195	continuous curvature with a new peak starting at -1.90V, above 80 ppm		
$LiClO_4$	0-10	0.9985	7.010	0-20	0.9994	5.371
	32-560	0.9997	1.020	continuous curvature		
K_2HPO_4	0-45	0.9992	3.972	2-32	0.9976	4.612
	50-106	0.9990	2.739	57-106	0.9968	2.992
	120-400	0.9996	1.994	120-400	0.9997	1.458
NaOAc	32-422	0.9990	1.273	continuous curvature		
HOAc/NaOAc (pH = 7.4)	32-539	0.9995	1.014	continuous curvature		
NH_3/NH_4Cl	0-32	0.9987	4.228	0-25	0.9998	5.472
(pH = 9.0)	32-130	0.9994	2.785	32-106	0.9988	3.005
	124-320	0.9996	2.159	32-249	0.9986	2.634
K_3. Citrate/	0-20	0.9975	6.342	0-20	0.9996	7.553
K_2H. Citrate	57-130	0.9994	1.593	---		
(pH = 8.0)	160-494	0.9994	0.961	63-267	0.9993	1.228
HCl (2M)	32-300	0.9994	0.498	---		

* The points used for each calibration plot were 7 to 10.
* The current was 2 and 10 µA for low and high concentrations respectively and the scan rate was 10mV/s.

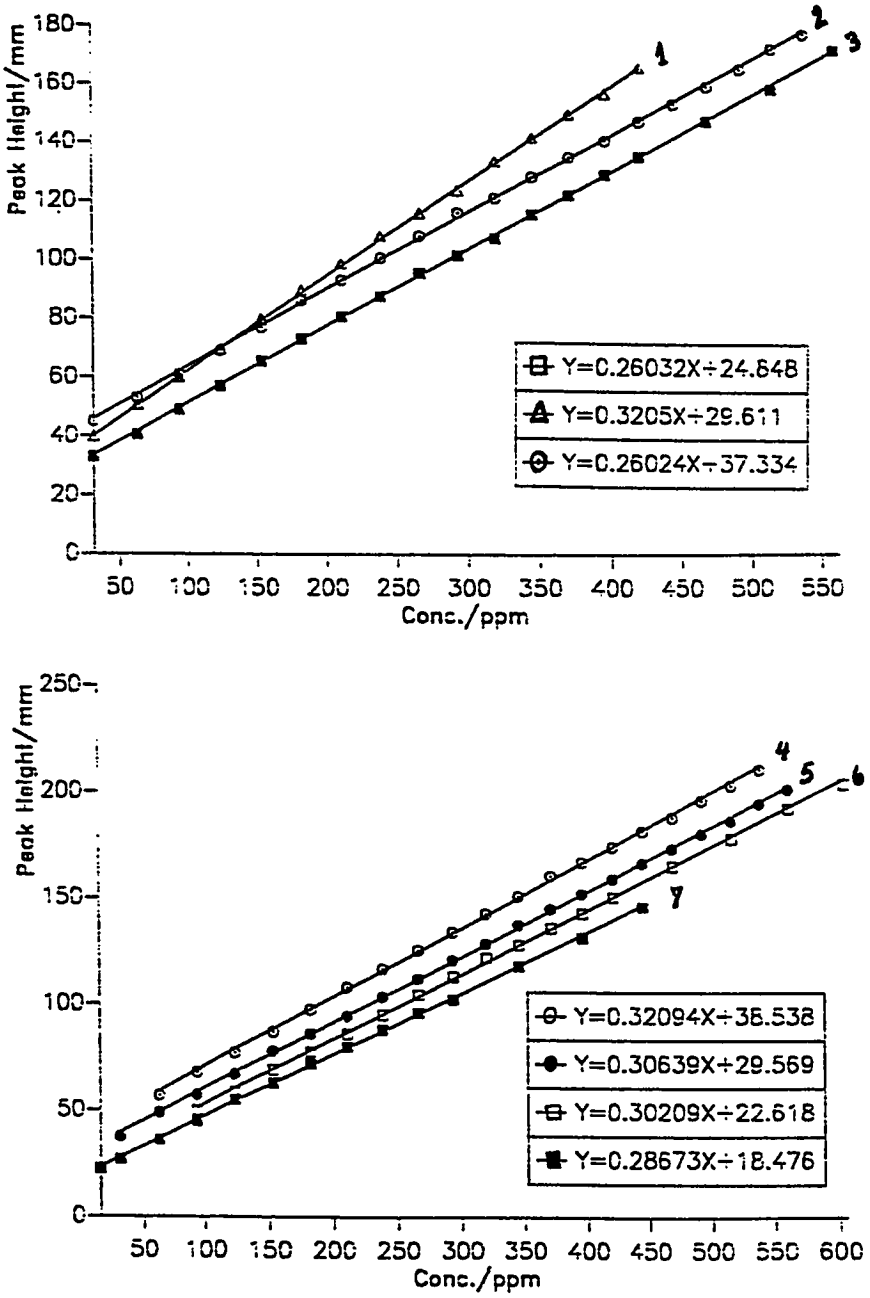


Fig. 2.8 Calibration plots for norfloxacin in different base electrolytes of 0.1 M concentration.
(1) NaOAc (2) LiCl (3) KCl (4) K_3 Citrate
(5) NaOAc/A. Acid (6) $K_2B_4O_7$ (7) $(CH_3)_4$ NBr.

The calibration behaviour of norfloxacin in hydrochloric acid (2M) based on the single wave observed has been also checked and found to be linear within the studied range, namely, 32 to 300 ppm ($1x10^{-4}$ to $9x10^{-4}$ M) norfloxacin (Table 2.3) with a nonsignificant shift in the peak potential.

2.4 Determination of Norfloxacin in Noroxin Tablets

2.4.1 Using the External Standards Calibration Method

Noroxin tablets were assayed by this method after grinding and dissolving in DMF according to the procedure mentioned in Section 2.2.3. The results are shown in Table 2.4. The table shows good recoveries (an average of 99.47% recovery and a relative standard deviation, RSD, of 2.49%) for the base electrolytes tested including the 2M hydrochloric acid. It is obvious that the drug excipents and the slight turbidity in the sample solutions did not affect the polarographic behaviour of norfloxacin.

2.4.2 Using the Standard Addition Method

The suitability of the method for the determination of Norfloxacin in Noroxin tablets is illustrated in Figures 2.9 and 2.10 and Table 2.5. An average of six successive standard additions to commercially obtained samples of formulated tablets resulted in well defined peaks. The Norfloxacin peak current in the original sample could thus be quantified based on the resulting standard addition plots shown in figures 2.9 and 2.10. Six consecutive analyses in K_3citrate and $K_2Br_4O_7$ base electrolytes yielded an average recovery value of 100.65% with a standard deviation of

Table 2.4: Determination of norfloxacin and nalidixic acid in Noroxin and Negram tablets respectively using the external standards calibration method.

Base Elec.	Range ppm	Corr. Coeff.	Quantity added ppm	Quantity found ppm	Recovery %
I. Norfloxacin					
$K_2B_4O_7$	3-12	0.9997	9.98	9.72	97.40
	3-32	0.9998	7.98	8.11	101.6
	6-124	0.9993	59.12	61.79	104.5
			97.57	99.11	101.6
	63-345	0.9995	197.73	199.72	101.0
K_3 Citrate	63-153	0.9990	62.70	59.53	94.94
			93.14	91.21	97.93
			122.99	120.80	98.22
			137.70	133.50	96.95
	60-500	0.9990	190.49	187.00	98.17
			296.31	300.00	101.2
			386.42	389.86	98.34
NaOAc[b]	32-125	0.9978	31.66	31.29	99.69
			62.70	62.91	100.3
			93.14	91.02	97.72
			122.99	114.9	93.40
HCl (2M)	32-300	0.9994	31.66	31.68	100.1
			42.26	43.54	103.0
			62.70	61.73	98.50
			93.14	93.37	100.2
			152.28	153.47	100.8
			181.00	180.36	99.60
			236.87	237.30	100.2
			264.04	268.94	101.9
				Average	99.47
				RSD	2.49%
II. Nalidixic Acid					
NaOAc (peak at -1.32 v)					
	20-90	0.9993	23.01	23.53	102.3
			34.34	34.49	100.4
			45.56	45.46	99.78
			67.68	68.85	101.7
			89.37	90.78	101.6
K_3 Citrate (peak at -1.52)					
	34-100	0.9958	34.34	35.41	103.1
			45.56	45.90	100.7
			67.68	68.02	100.5
			89.37	91.08	101.9
			100.1	102.6	102.1
HCl, 2M (peak at -0.78 V)					
	10-110	0.9992	11.56	11.75	101.6
			34.34	35.07	102.0
			45.56	45.63	100.2
			67.68	68.39	101.0
			89.37	88.94	99.50
				Average	100%
				RSD	1.01%

a: According to the nominal value reported by the manufacturers.
b: Peak D was used here. Peak C gave a very high recovery.

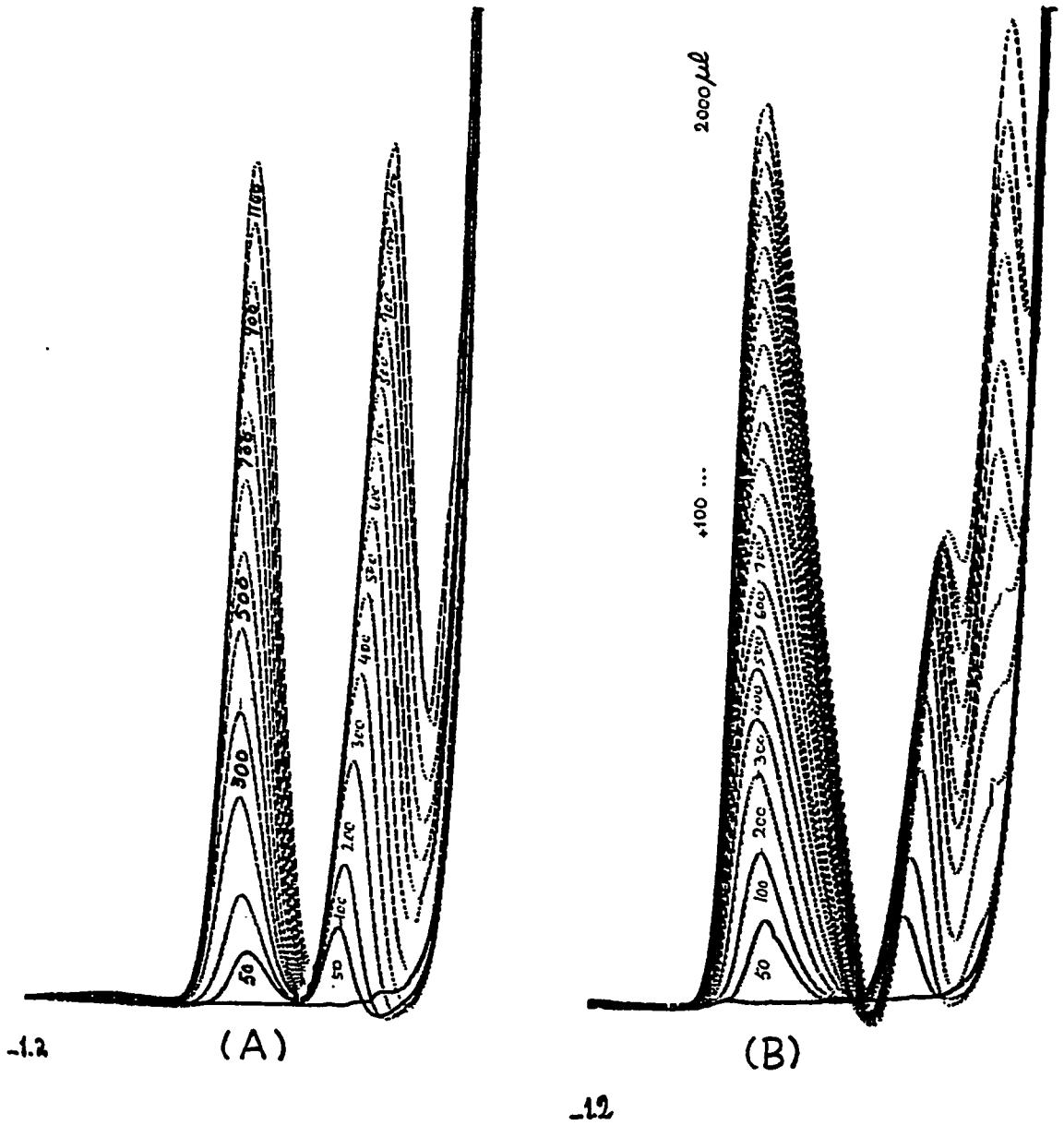


Fig. 2.9 Determination of linearity of calibration plots for norfloxacin by using dpp, (A) in 0.1M $K_2B_4O_7$ base electrolyte and in (B) 0.1M K_3 Citrate base electrolyte. Norfloxacin concentration checked was in the range 16 ppm (5×10^{-5} M) to 321 ppm (1×10^{-3} M) in case (A) and from 16 ppm to 540 ppm (1.7×10^{-3}M) in case B. Scan rate used was 5 mV/sec and current sensitivity was 10 µA.

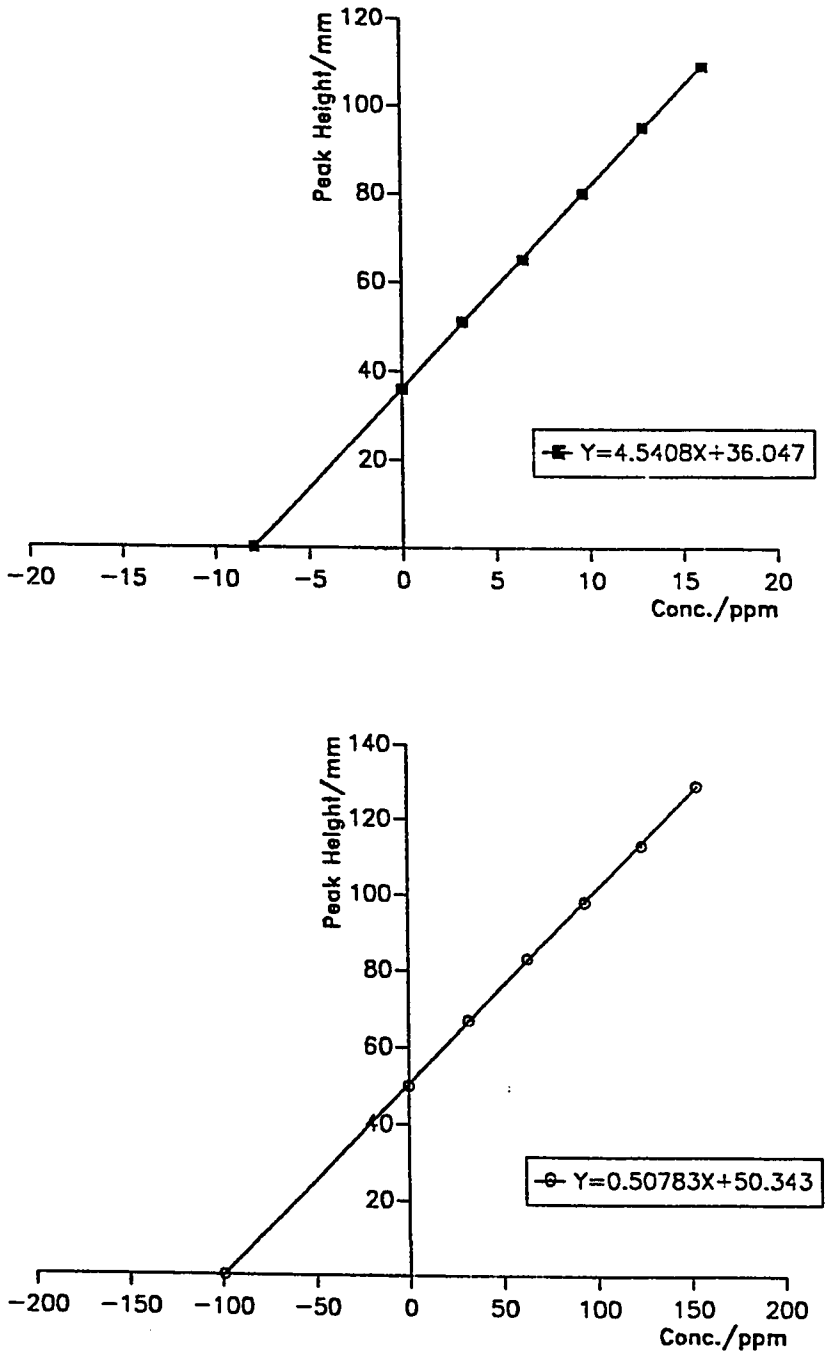


Fig. 2.10 Standard addition plots for the determination of norfloxacin in Noroxin tablets in 0.1M K_3 citrate base electrolyte. The scan rate used was 5mV/sec and the sensitivity was 2μA.

Table 2.5: Determination of norfloxacin in Noroxin tablets using the standard additions method. Wave C was used for analysis. Scan rate of 5mV/sec and current sensitivity of 2μA were used.

Base Electrolyte	Number of Data Points n	Correlation Coefficient	Sens.($x10^{-2}$) $\mu A.L.mg^{-1}$	Quantity added ppm	Quantity found ppm	Recovery %
K_3 Citrate	6	0.9999	3.575	7.97	7.94	99.62
	6	0.9996	3.272	19.89	19.72	99.15
	5	0.9999	2.536	59.02	59.53	100.86
	6	0.9999	2.000	97.41	99.13	101.77
$K_2Br_4O_7$	7	0.9970	3.543	7.97	7.84	98.37
	6	0.9999	3.364	19.89	20.71	104.12
					Average R.S.D.	100.65 2.09

2.09%. The excipients in the tablets did not interfere in the analysis. Hence, these results confirm the utility of this proposed method in the determination of norfloxacin in formulated tablets.

2.5 Determination of Nalidixic Acid as Individual Component in Tablets and in the Presence of Norfloxacin as Interferent

The possibility of determining nalidixic acid as the individual component in tablets and in the presence of norfloxacin has been investigated here. Figs. 2.11 and 2.12 show the dp polarograms for norfloxacin, nalidixic acid and mixtures of them in hydrochloric acid (2M) and sodium acetate (0.1M) base electrolytes respectively. Nalidixic acid showed two dp reduction waves in the 2M HCl base electrolyte at -0.78V and -1.01V for 4.3×10^{-5}M nalidixic acid with a little shift with concentration. These two waves were close to $E_{1/2}$ values reported for nalidixic acid (26). Using sodium acetate or tripotassium citrate as the base electrolyte, three reduction waves at -1.32V, -1.52V, and -1.78V have been observed for 4.3×10^{-5} M nalidixic acid. These potentials showed shifts in the range of $\pm$ 80 mV for nalidixic acid concentrations reaching up to 4.8×10^{-4}M. These dp peak potentials correspond to $E_{1/2}$ values of the three dc waves reported for nalidixic acid at similar pH values. Nalidixic acid as the individual component in the sample solution was then determined in Negram commercial tablets and gave good recoveries (an average of 101.2% recovery and RSD of 1.01%) as observed from Table 2.4.

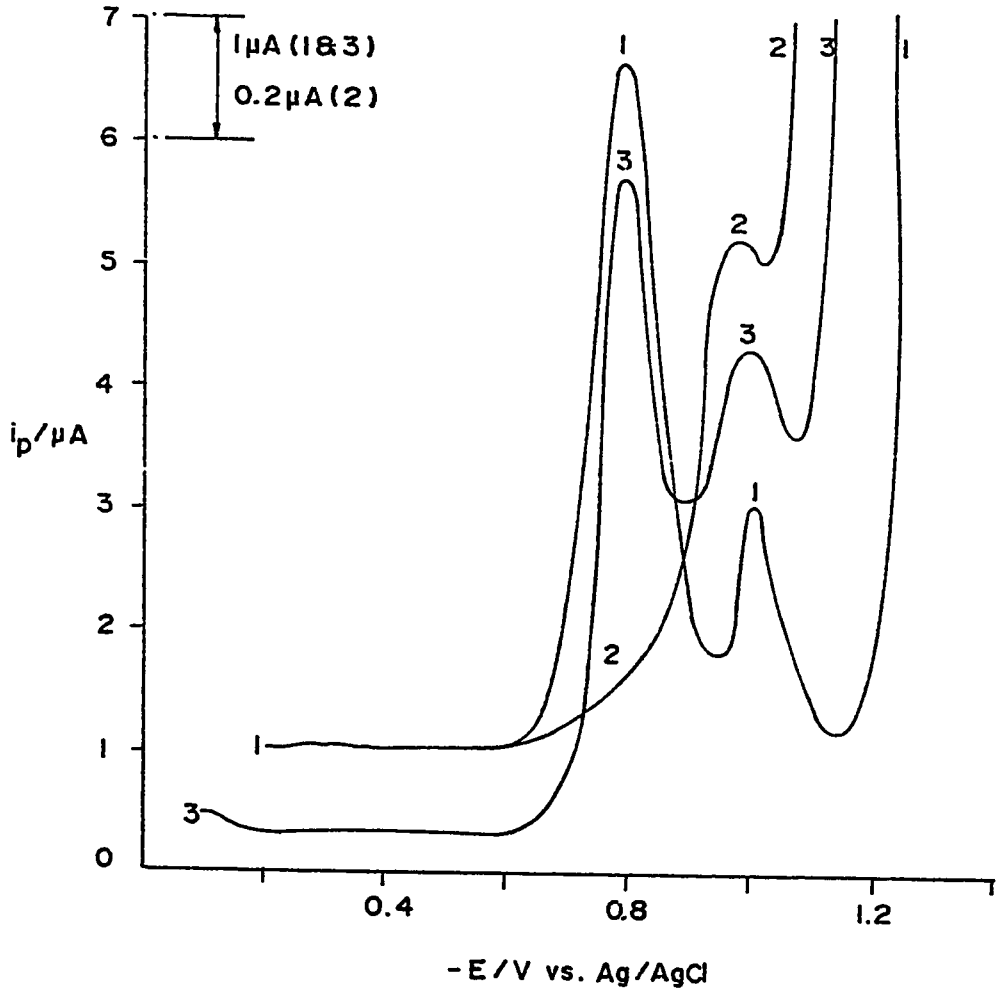


Fig. 2.11 Dp polarograms for $4.8X10^{-4}$ M nalidixic acid(polarogram 1), $4.8X10^{-4}$M norfloxacin (polarogram 2) and a mixture of $4.8X10^{-4}$ M of each(polarogram 3) in 2M HCl base electrolyte.

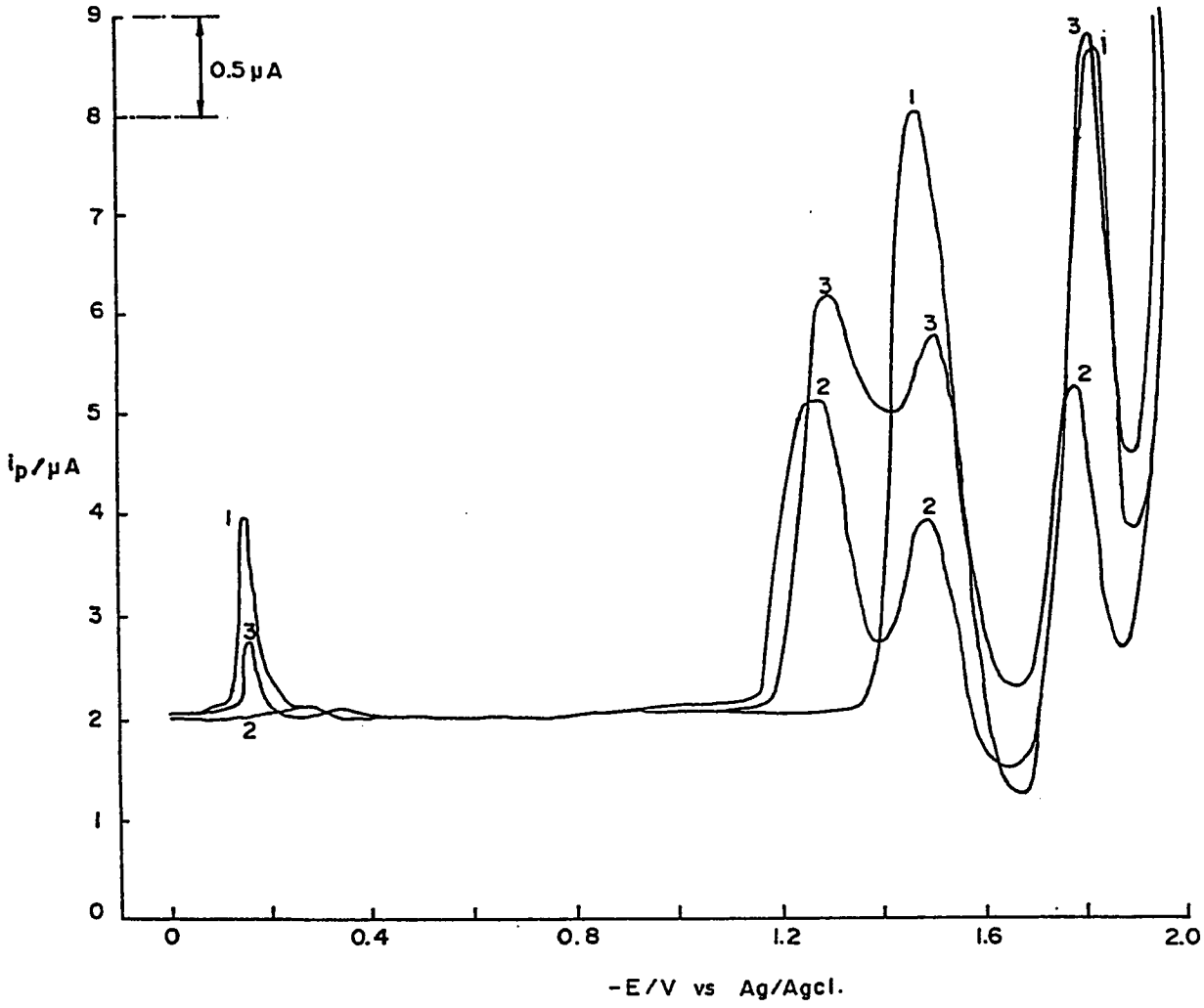


Fig. 2.12 Dp polarograms for $2.9X10^{-4}$ M norfloxacin(polarogram 1), $2.9X10^{-4}$ M nalidixic acid (polarogram 2) and a mixture of $2.9X10^{-4}$ M of each(polarogram 3) in 0.1M NaOAc base electrolyte.

Figs. 2.11 and 2.12 show also that the reduction waves for either norfloxacin or nalidixic acid in mixtures appear almost in the same positions as those for the individual components. Incremental additions of nalidixic acid to a sample solution of norfloxacin in sodium acetate or tripotassium citrate base electrolytes have shown a significant effect on the dp peak at -1.32V. This peak belongs only to nalidixic acid, thus it was chosen for determination of nalidixic acid in the presence of norfloxacin (Table 2.6).

Nalidixic acid showed two dp peaks at -0.78V and -1.01V in 2M HCl base electrolyte. Meanwhile, norfloxacin showed only a peak at -1.02V. Incremental additions of nalidixic acid to a mixture of the two affected the peak at -0.78V significantly. Thus, the peak at -0.78V was used for nalidixic acid determination in the presence of norfloxacin (Table 2.6). Good recoveries for nalidixic acid (an average of 100.3% recovery and RSD of 1.8%) have been obtained using the external standards calibration method (Table 2.6).

2.6 Determination of Norfloxacin in Noroxin Tablets in the Presence of Nalidixic Adic as Interferent

Fig. 2.13 shows the dp polarograms for norfloxacin in the presence of 45.56 ppm nalidixic acid in sodium acetate and tripotassium citrate as base electrolytes. Incremental additions of norfloxacin showed a significant effect on wave C at -1.52V which is used for the analysis of the formulated tablets. Nalidixic acid is added for both the standards and the

Table 2.6 : Determination of nalidixic acid in Negram in the presence of 60 ppm (1.9×10^{-4} M) norfloxacin, using the external standards calibration method. Same quantity of norfloxacin was added to both standard and unknown sample solutions.

Base Elec.	Range ppm	Corr. Coeff.	Nalid. acid added[a] ppm	Nalid. acid found ppm	Recovery %
NaOAc					
	22-90	0.9993	21.92	21.08	96.12
			43.84	44.92	102.5
			65.76	65.36	99.39
K_3 Citrate					
	22-88	0.9965	32.88	33.18	100.9
			43.84	43.77	99.84
			65.76	66.13	100.6
			87.68	87.31	99.58
HCl (2M)					
	22-77	0.9991	21.92	22.38	102.1
			32.88	33.57	102.1
			43.84	43.48	99.20
			54.80	54.31	99.10
			65.76	66.98	101.9
				Average	100.3
				RSD.	1.80%

a: According to the nominal value reported by the manufacturers.

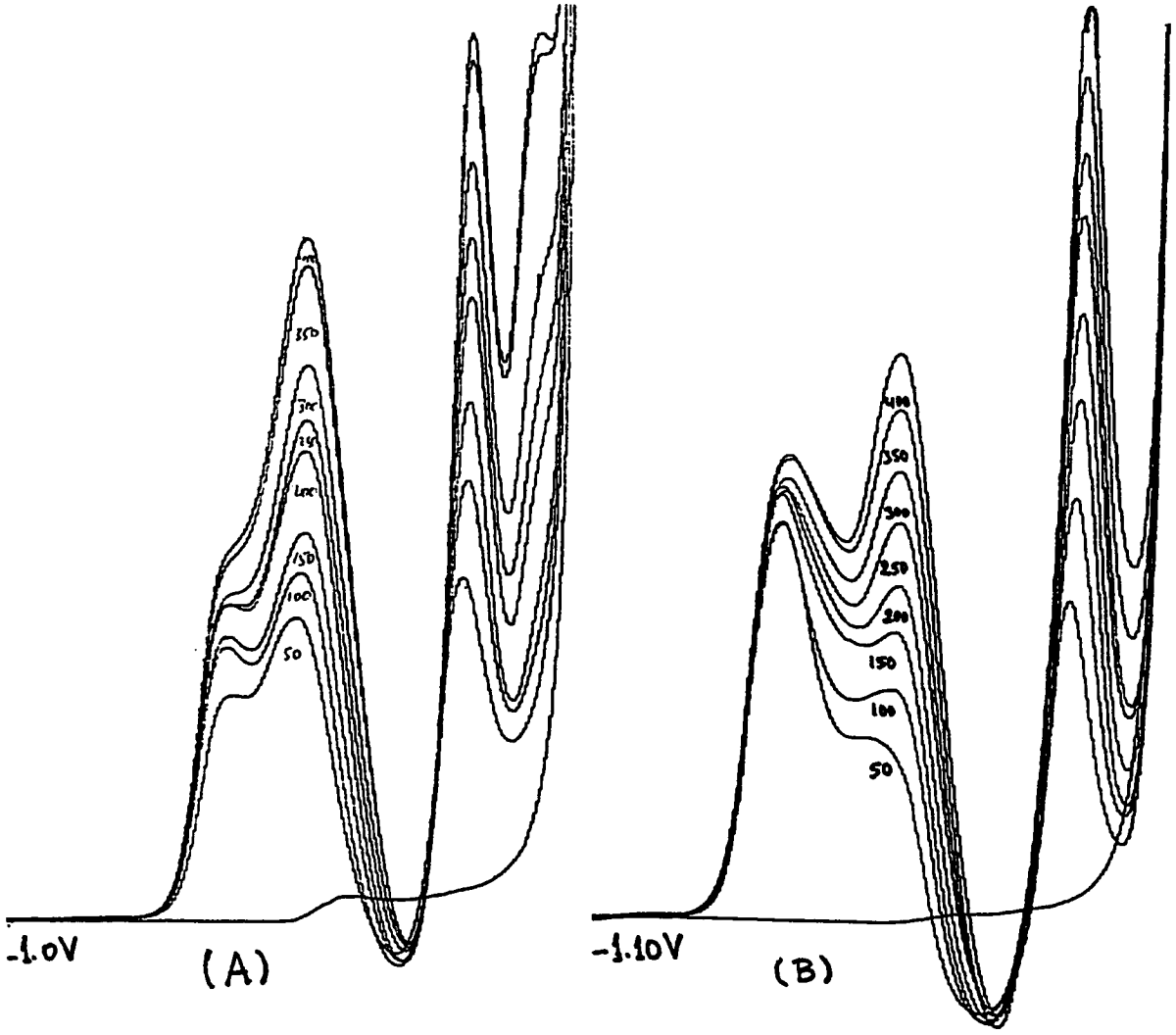


Fig. 2.13 Dp polarograms for norfloxacin in the presence of 45.56 ppm nalidixic acid in (A) 0.1M K_3 citrate base electrolyte and in (b) 0.1M NaOAc base electrolyte. Scan rate used was 10 mV/sec and current sensitivity was 2μA.

unknowns. Good recoveries for norfloxacin (an average of 99.80 with an RSD of 2.33%) have been obtained using the external standards calibration method (Table 2.7). From the results, we observe that the drug additives, and the nalidixic acid added did not interfere in the analysis.

Table 2.7: Determination of norfloxacin in noroxin tablets in the presence of 45.56 ppm (1.96×10^{-4} M) nalidixic acid, using the external standards calibration method. The samve quantity of nalidixic acid is added to both standard and unknown sample solutions. Wave C at -1.52v was used for analysis. Scan rate of 10mv/sec and current sensitivity of 2μA were used.

Base Electrolyte	Range (ppm)	Correlation Coefficient	Sens.($\times 10^{-2}$) μA.L.mg^{-1}	Quantity added (ppm)	Quantity found (ppm)	Recovery %
K_3 Citrate	0 - 121	0.9960	1.60	45.25	43.39	96.0
				60.34	60.65	100.5
				90.50	88.38	97.7
				124.53	124.11	99.7
NaOAc	0 - 121	0.9995	2.34	30.17	30.40	101.07
				60.34	60.62	100.4
				90.50	93.36	103.16
					Average	99.79
					R.S.D.	2.33

2.7 Analytical Approach to the Determination of Equimixtures of Norfloxacin and Nalidixic Acid

Figs. 2.14 and 2.15 show the dp polarograms and the calibration plots for 1:1 mixture solution of nalidixic acid and norfloxacin in sodium acetate and HCl (2M) as base electrolytes. In sodium acetate, nalidixic acid showed three distinct peaks at -1.32V, -1.52V and at -1.78V. However, norfloxacin showed only two peaks at the same potentials as the 2nd and the 3rd peaks of nalidixic acid. Thus, the first peak at -1.32V belongs to nalidixic acid only and can be used as a characteristic peak in qualitative and quantitative analysis. The linearity was tested for this peak for nalidixic acid concentrations in the range of 2.34 - 270 ppm (1.00×10^{-5} to 1.16×10^{-3} M) and found to be linear with a correlation coefficient of 0.9994 and a sensitivity of 0.0419 $\mu A.L.mg^{-1}$ (Table 2.8). However, nalidixic acid in HCl (2M) showed two peaks only at -0.78V and at -1.01V, meanwhile norfloxacin showed one peak only at -1.02V. Hence, the first peak of nalidixic acid in this base electrolyte can be used for qualitative and quantitative analysis. Moreover, the linearity was tested using this peak for nalidixic acid in the range 2.34-200 ppm (1.00×10^{-5} M to 8.61×10^{-4} M) and found to be linear with a correlation coefficient of 0.9993 and a sensitivity of 0.0451 $\mu A.L.m^{-1}g$. (Table 2.8)

We conclude that we can determine nalidixic acid qualitatively and quantitatively in a 1:1 mixtures with norfloxacin without serious interference effect.

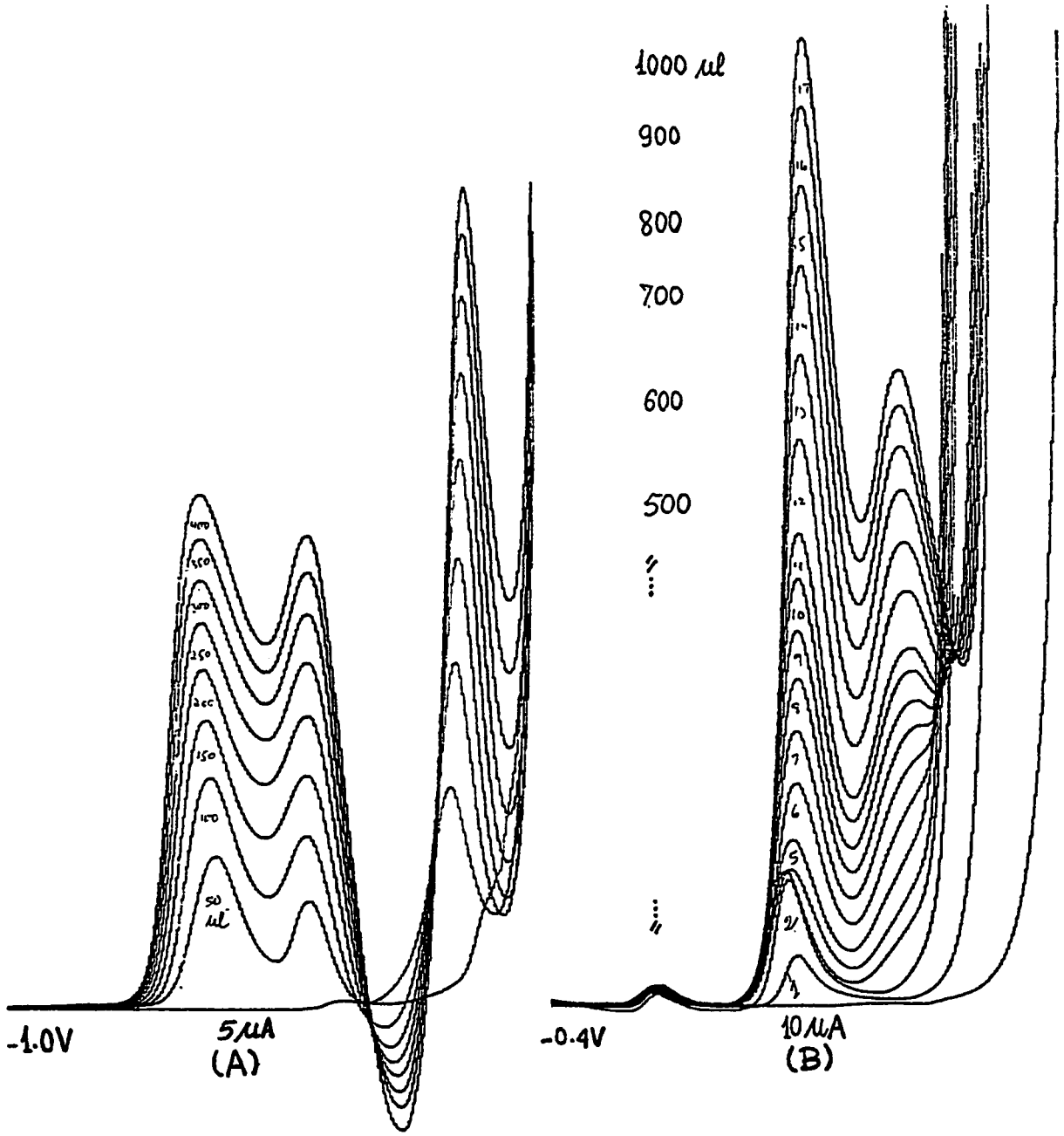


Fig. 2.14 Dp polarograms for a 1:1 mixture solution of norfloxacin and nalidixic acid in (A) 0.1M NaOAc base electrolyte and in (B) 2M HCl base electrolyte. Scan rate used was 10 mV/sec. Current sensitivity used was 5µA with NaOAc and 10µA with HCl (2M) base electrolytes.

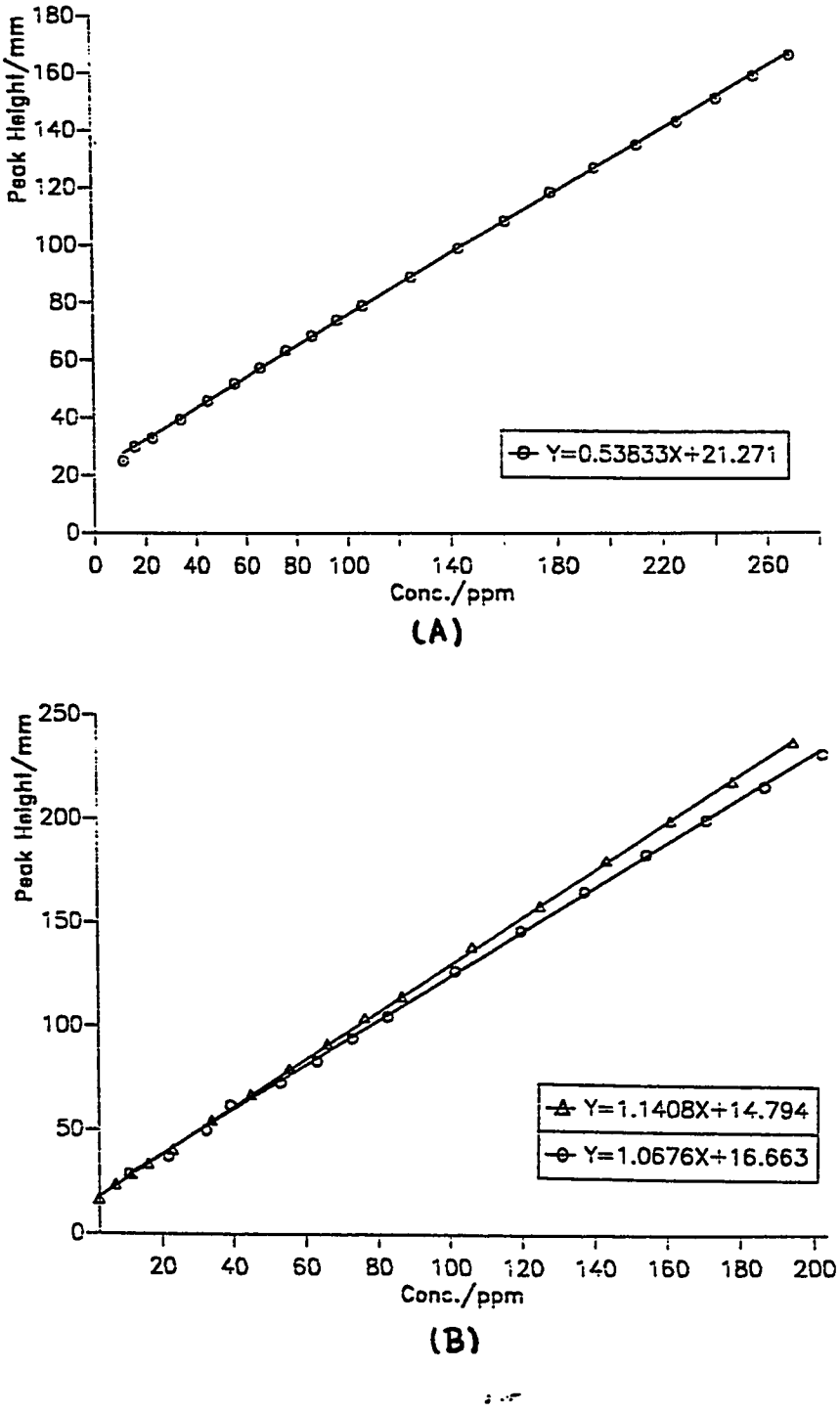


Fig. 2.15 Calibration plots for nalidixic acid in 1:1 mixture solution with norfloxacin in (A) 0.1 M NaOAc base electrolyte and in (B) 2M HCl base electrolyte. Scan rate used was 1 0 mV/sec and sensitivity was 5μA.

Table 2.8: Analytical assessment of equimixtures of norfloxacin and nalidixic acid in sodium acetate and HCl (2M). Scan rate of 10mV/sec and sensitivity of 5μA, were used.

Base Electrolyte	$-E_p$ used V	Range ppm	Correlation Coefficient	Sens. x 10^{-2} $\mu AL.mg^{-1}$
NaOAc	-1.32 / -1.30	2.34 - 270	0.9994	4.185
HCl 2M	-0.78 / 0.82	2.34 - 200	0.9993	4.510

CHAPTER THREE

DETERMINATION OF ULTRATRACE LEVELS OF NORFLOXACIN BY ADSORPTIVE - CATALYTIC STRIPPING VOLTAMMETRY

3.1 Adsorptive Stripping Voltammetry as Analytical Technique for Ultra Trace Analysis

Some anions or organic compounds can be accumulated on a mercury electrode to form an insoluble compound with the mercury ions obtained by dissolution of the mercury electrode at positive potentials. In this type of cathodic stripping voltammetry (CSV), the reduction process of the mercury compound on the electrode surface is studied. The most important step, leading to a substantial increase in the sensitivity is electrolytic accumulation of the species on the working electrode (36). Some other principles, can be used for accumulation of the substance to be determined (36). One of these is adsorption, which is characterized by the nonelectrolytic nature of accumulation process. This preconcentration step forms the basis for the adsorptive stripping voltammetry (AdSV). At a given temperature, the amount of substance adsorbed is dependent on its concentration in solution. The velocity of formation of the adsorbed layer is affected by both, the rate of the actual adsorption of the substance from the solution layer in direct contact with the electrode and the rate of

diffusion of the substance from the bulk of the solution to the electrode surface. The slower step of the two processes is considered as the rate controlling step in the formation of the adsorbate (36). Finally, adsorption equilibrium is established between the concentration in the solution and that on the surface of the electrode.

Some forms of polarographically active substances are adsorbed at the mercury dropping electrode. In such instances two waves may be observed on polarographic curves, one of which corresponds to the reduction of the free form and the other to the substance in the adsorbed state (8) as observed from Fig. 1.10. The adsorption current increases with the increasing concentration of the electroactive species but only to a certain extent depending upon the surface of the electrode. The adsorption energy causes a shift in the half-wave potentials to more positive or negative values (8). The adsorption peak is shifted to more negative potential values with increasing amount of the adsorbed compound at the electrode surface (37).

The present study describes an extremely sensitive adsorption stripping voltammetric procedure for ultratrace determination of norfloxacin based on the coupling of catalytic and adsorption processes. The dual current-magnifying effect associated with the adsorptive catalytic stripping scheme offers remarkable sensitivity, with detection limit at the picomolar (sub-ppt) level following a short (1 min) accumulation time (38, 39, 40)

Catalytic currents described as regeneration currents, increase with concentration and reach a limiting value with the increasing concentration

of the catalytically active substance. Most catalytic currents increase with decreasing pH and with the increasing concentration of the buffer solution used. Some catalytic currents are also sensitive to the ionic strength. Typical examples of such catalytic currents occur in the presence of different organic heterocyclic nitrogen compounds, some weak acids and bases in buffers, and occur with hydroquinones (8). The mechanism of most reactions which cause catalytic currents, has not been determined with certainty, but some observations show that a different mechanism occurs with different substances. In some instances, the adsorption of the catalyst and possibly of the buffer together with the motion of the solution are claimed to play an important role (8).

3.2 Experimental Procedures

3.2.1 Apparatus

An EG & G PAR polarographic analyzer Model 174A was used in connection with the EG & G PAR Model 303 static mercury drop electrode serving as the working electrode. A medium-size hanging mercury drop electrode (HDME), with an area of 0.016 cm^2, was employed. Data were displayed on an EG & G PAR Model 0074 X-Y recorder. A saturated Ag/AgCl electrode was used as the reference electrode and a platinum wire electrode was used as the auxiliary electrode. However, an EG & G PAR voltametric analyzer Model 264A was used in connection with the EG &G PAR Model 303A static mercury drop electrode to record the cyclic voltammograms. The Data were displayed on an EG & G PAR Model 0150 X-Y recorder.

3.2.2 Analytical Procedures

A 0.01014M stock solution of norfloxacin was prepared by dissolving an appropriate amount of norfloxacin in 50 ml of dimethyl formamide then diluted to 100 ml with the same solvent. Aliquots of this solution were diluted as required.

A 10 ml volume of 0.1 M supporting electrolyte solution was pipetted into the cell and purged with nitrogen-free oxygen for 12 min. increments of the stock solutions of norfloxacin prepared in dimethyl formamide were spiked into the base electrolyte. With each addition, the solution was deaerated for 1 min. The preconcentration potential measured versus the Ag/AgCl reference electrode was applied to a fresh hanging mercury drop while the solution is being stirred. Following the accumulation period, the stirring was stopped for 20 sec as the equilibration time after which the voltammogram was recorded by applying a negative-going differential pulse scan with an amplitude of 50 mV and an appropriate scan rate for each system. The limiting currents were measured and calibration curves in several base electrolytes were constructed. The measurements were carried out at room temperature ($25 \pm 1^{\circ}C$).

3.2.3 Preparing of Norfloxacin Tablets for Analysis

Eight tablets of Noroxin were ground to a fine powder in a mortar. An accurately weighted portion of the powder equivalent to about 400 mg of norfloxacin was dissolved in DMF, transferred into a 100 ml

volummetric flask and diluted to the mark with DMF. The solution was slightly turbid but no further treatment was made. Known volumes of the diluted norfloxacin standard solutions were spiked into 10 ml aliquots of base electrolyte. Throughout this operation, with each addition, nitrogen was purged through the solution for 1 min.

3.3 Results and Discussion

3.3.1 Preliminary Adsorptive Studies for Norfloxacin in Various base Electrolytes

The spontaneous accumulation of norfloxacin and the catalytic character of its redox process are evident from the cyclic voltammogram and the differential pulse adsorptive stripping voltammograms shown in Figs. 3.1 and 3.2. From the cyclic voltammogram, one cathodic peak is observed in the forward scan in the range -1.48V - 1.63V. This peak was previously (chapter 2) attributed to the reduction of the ethylenic bond in the azinone ring. Scanning in the reverse direction produced a cathodic peak in the range -1.32V to -1.68V, which is indicative of a catalytic process. The response increases dramatically when an accumulation period precedes the potential scan. (Fig. 3.3) indicating enhancement by adsorption.

The strong adsorption of norfloxacin at the hanging mercury drop electrode can be used as an effective preconcentration step, prior to the voltammetric measurement, making possible an extremely sensitive determination. For example, Fig. 3.2 shows differential pulse voltammo-

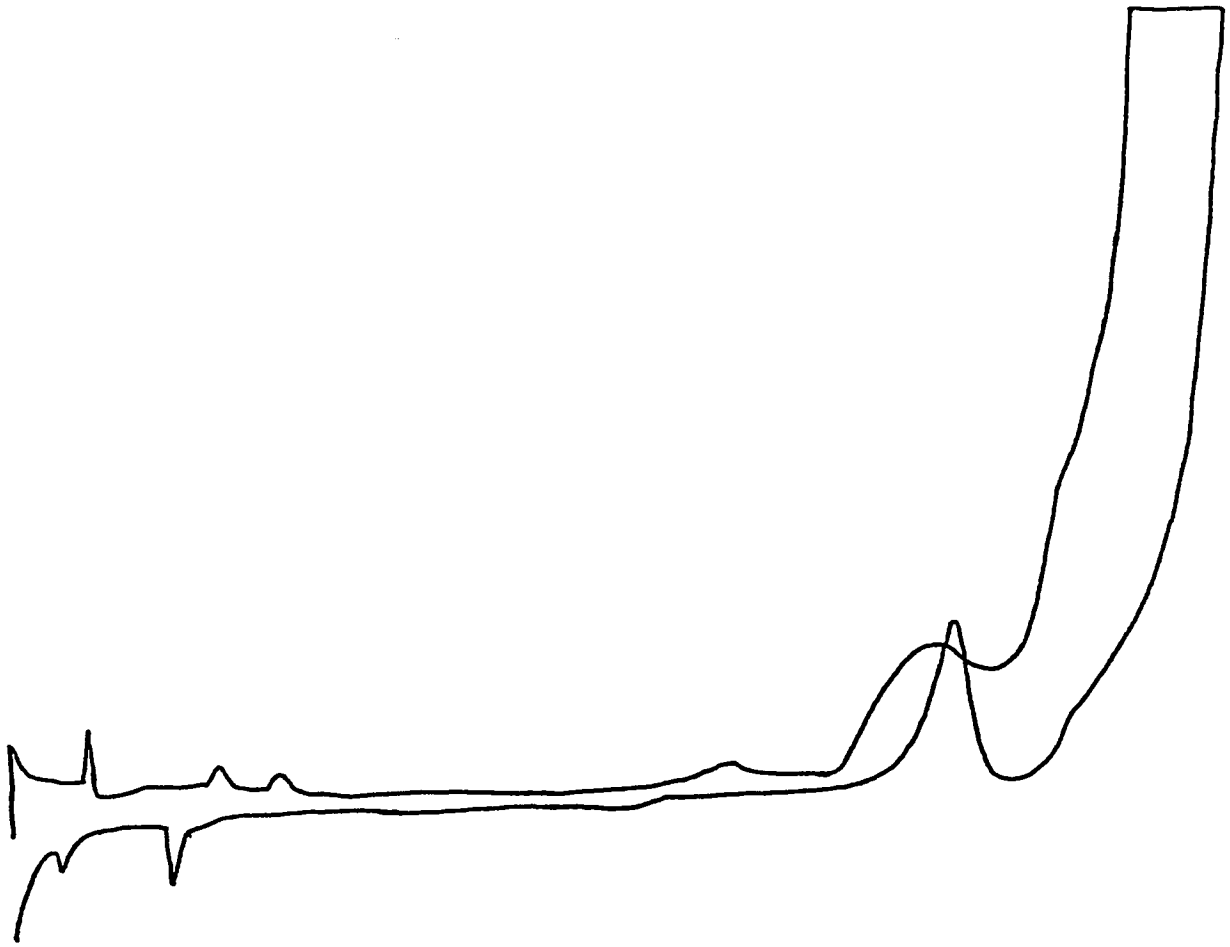


Figure 3.1 Cyclic voltammogram for 1x10-4 M norfloxacin in 0.1M LiCl base electrolyte. Scan rate used was 50mV/sec, current sensitivity was 2μA and peak amplitude was 50mV.

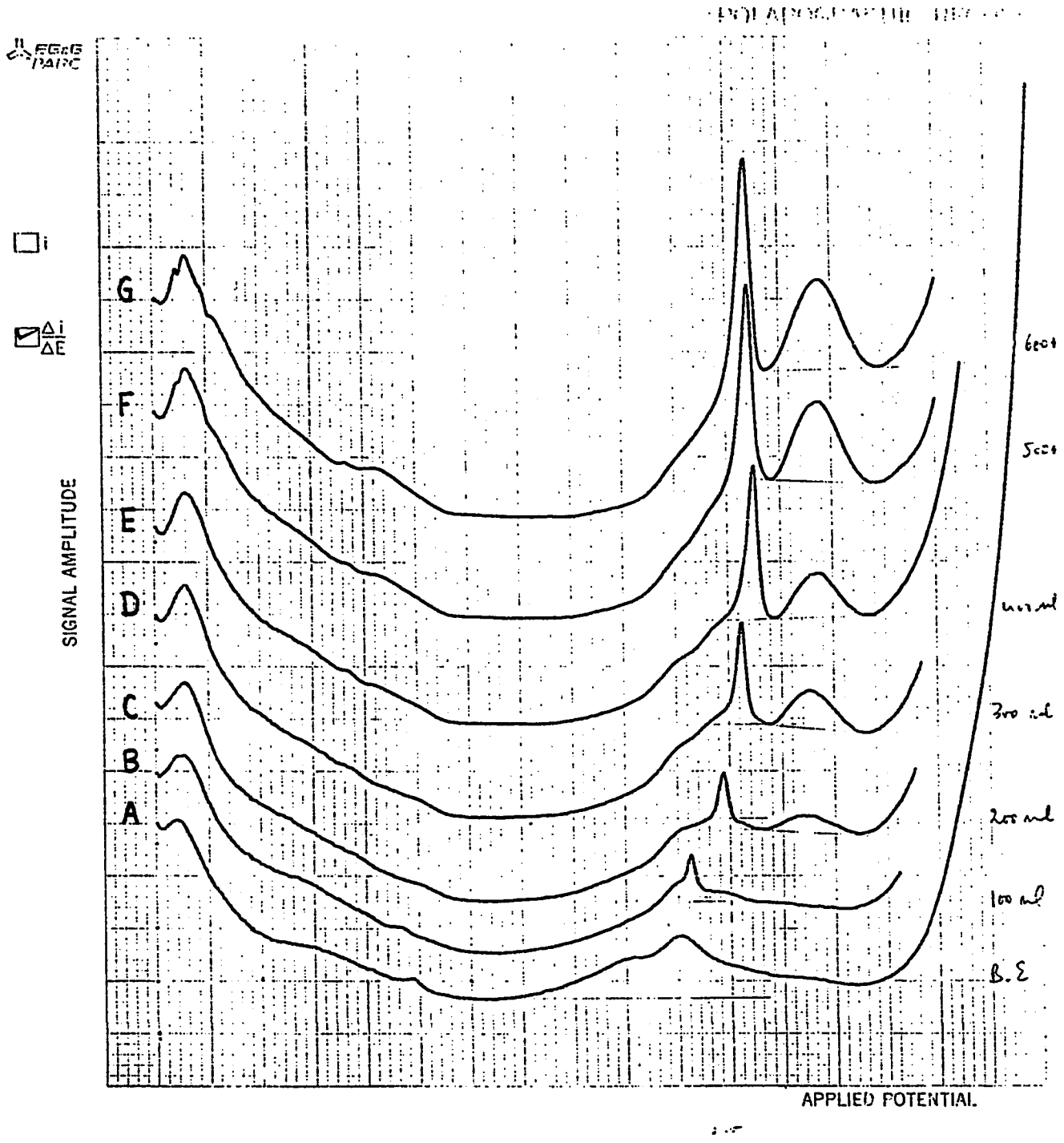


Figure 3.2 Dp stripping voltammograms for: 0 (A), 0.32 (B), 0.63 (C), 0.94 (D), 1.24 (E), 1.54 (F) and 1.83 (G) ng/l of norfloxacin in 0.1M LiCl base electrolyte under the optimum conditions of scan rate, accumulation time and accumulation potential (Table 3.2). Current sensitivity used was 1μA and peak amplitude was 50mV.

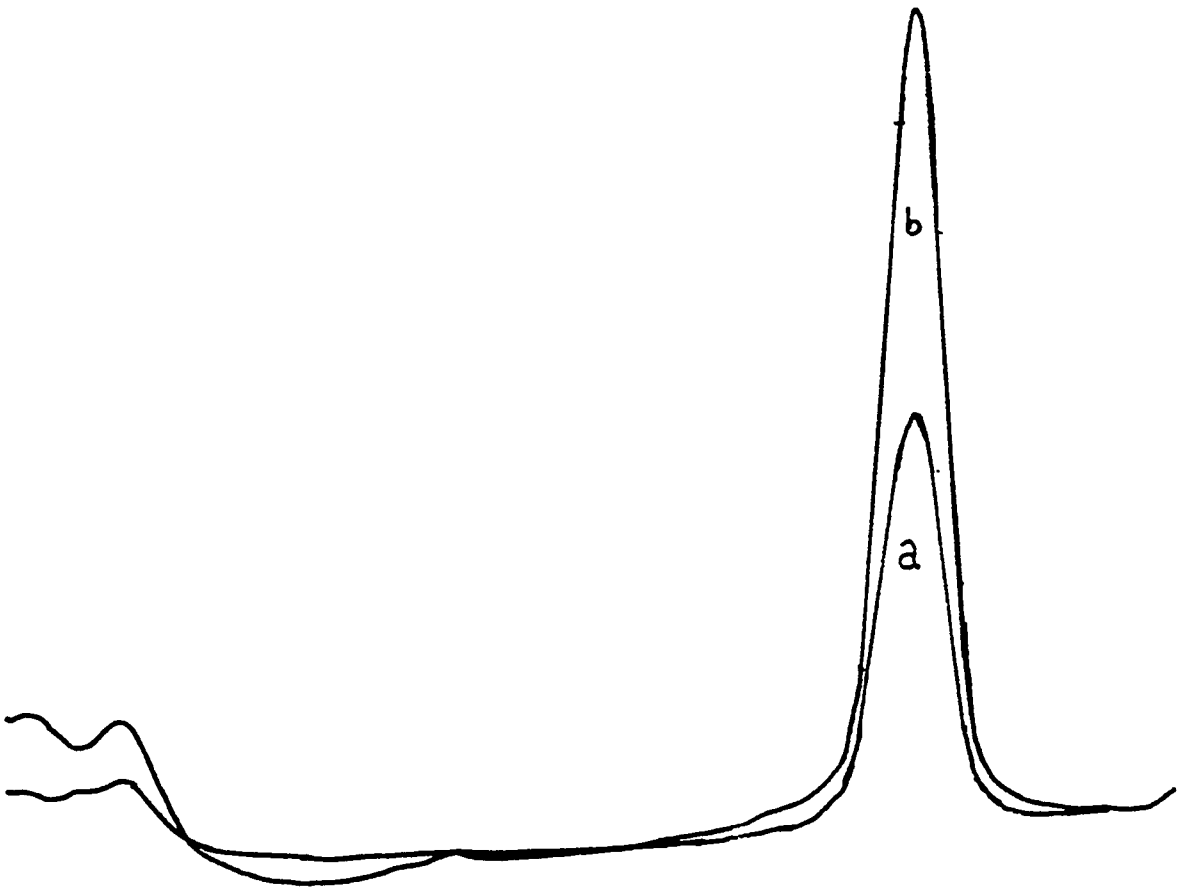


Figure 3.3 Dp voltammograms for 32.06 ug/l of norfloxacin in 0.1M LiCl base electrolyte following 1 min (a) and 4 min (b) accumulation periods. Scan rate used was 2mV/sec and current sensitivity was 2μA.

grams of extremely diluted solutions of norfloxacin (1.00×10^{-12} M, or 0.321 ng/l), after a preconcentration period of 4 min in LiCl. As a result, convenient measurement of parts per trillion norfloxacin were possible.

The differential pulse stripping voltammetry of norfloxacin in different base electrolytes showed three defined peaks in the ranges -1.02V to -1.18V, -1.21V to -1.34V and -1.40V to -1.54V. Table 3.1 summarizes the AdSV behavior of norfloxacin in various supporting electrolytes. The main peak is the one appeared in the range of -1.02V to -1.18V and it is the most sensitive peak. Thus, it was used for the determination of norfloxacin in Noroxin tablets.

The three waves mentioned above were concentration dependent although a break or more in the linearity of the calibration curves was observed. In most cases, the two waves at the more negative potentials become more significant at higher concentrations. However, the first wave at the most positive potential was the most sensitive, and appeared even at extremely low concentrations.

AdSV is being used for the trace analysis of surface active compounds, but also non-surface active substances can be accumulated at the electrode surface after formation of complexes with surface-active ligands. A general problem during adsorptive stripping experiments is the interfering processes from the other solution constituents. Consequently, it is especially difficult to determine trace components in an electroactive matrix. The presence of coadsorption films on the electrode may inhibit or

Table 3.1: Adsorption stripping behavior of norfloxacin in various supporting electrolytes. The scan rate and current sensitivity were 10 mV/sec and 20μA respectively.

Base Electrolyte	$-E_{P_1}$ (V)	$-E_{P_2}$ (V)	$-E_{P_3}$ (V)	Description
1. LiCl	1.02/1.18	1.26/1.30	1.40/1.48	ip_1 : well defined peak ip_2 : ill-defined peak ip_3 : appeared as a shoulder
2. NaCl/NaOH pH = 8.80	1.06/1.07	1.21/1.23	1.45/1.53	ip_1 : well defined peak ip_2 : ill-defined peak ip_3 : appeared as a shoulder
3. NaOAc	1.05/1.07	1.341/36	---	ip_1 : well defined peak ip_2 : appeared as a shoulder ip_3 : -----
4. CH_3COOK	1.03/1.18	1.28/1.32	1.44/1.54	ip_1 : well defined peak ip_2 : ill-defined peak ip_3 : appeared as a shoulder
5. KCL	1.05/1.17	1.28/1.34	1.43/1.47	ip_1 : well defined peak ip_2 : ill-defined peak ip_3 : ill-defined peak
6. K_2HPO_4	1.06/1.13	1.20/1.28	1.45/1.51	ip_1 : low sensitivity ip_2 : ill-defined ip_3 : appeared as a shoulder
7. $K_2B_4O_7$	1.05/1.07	1.28/1.32	1.40/1.48	ip_1 : well defined peak ip_2 : ill-defined peak ip_3 : appeared as a shoulder

Table 3.1 (Continued)

Base Electrolyte	$-E_{P_1}$ (V)	$-E_{P_2}$ (V)	$-E_{P_3}$ (V)	Description
8. $LiClO_4$	1.06	---	1.46	i_{P_1} showed good sensitivity, but another scale appeared at - 1.12V which interferes with the peak of norfloxacin at - 1.06V. i_{P_3} was a shoulder and it was very close to the base electrolyte discharge curve.
9. $(CH_3)_4$ NBr	1.04	---	1.46	i_{P_1} showed lower sensitivity compared to the $LiClO_4$ case i_{P_3} was a flat shoulder, almost not seen.
10. NH_3/NH_4Cl pH = 9.00	1.08	---	1.44	i_{P_2} low sensitivity i_{P_3} appeared as a shoulder and it was too close to the base electrolyte discharge curve.
11. K_3 citrate/ K_2 citrate pH = 8.00	---	---	1.40	appeared as a shoulder.
12. K_3 citrate	---	---	1.48	ill-defined peak
13. Pot. tri-citrate	---	1.23	---	ill-defined peak
14. tri-ammonium citrate	1.04	---	---	ill-defined peak

retardate the electrode reactions, depending on the thickness of the adsorbed film. In the case of inhibition, no reduction peak of the electrolyte will be seen. However, in the case of retardation, a decreased peak height with shift to negative potential will be noticed (41). Retardation effect of surfactants varies from one to another depending on the chemical structure, and on the composition of the supporting electrolyte solution. Table 3.1, shows that norfloxacin gives peaks of low sensitivity that cannot be used for analytical purposes, and this may be ascribed to the coadsorption of the base electrolyte anions, as it is reported for the citrates which coadsorb very strongly (41) on the hanging mercury drop electrode.

For analytical purposes, it is important to note that the same fraction of the analyte ion is stripped during each experiment to give a certain current intensity. Therefore, it is essential that the electrode surface area, electrode volume, rate of solution stirring, and length of the preconcentration period to be as reproducible as possible to ensure that good precision will be obtained.

3.3.2 Parameters Affecting the Adsorptive Stripping Voltammetric Behavior of Norfloxacin at HMDE

In order to optimize the accumulation conditions, the effect of base electrolyte, accumulation time, accumulation potential and scan rate were studied at a fixed pulse amplitude of 50 mV and equilibration time of 20 sec. The results are indicated in Table 3.2.

Table 3.2: Optimum accumulation potential (E_i), scan rate (V) and accumulation time (t_{acc}) for norfloxacin in various base electrolytes of (0.1M) determined at the main peak indicated. Norfloxacin concentration was 1 x 10^{-6}M, current sensitivity used was 2μA, deaeration time was 12 min and $t_{eq.}$ was 20 sec.

Base Electrolyte	-Ep (V)	-E_i (V)	V (mV/sec)	t_{acc} (min.)
1. LiCl	1.06	0.0	2.0	4.0
2. NaCl/NaOH pH 8.80	1.07	0.1	10.0	1.0
3. CH_3COONa	1.06	0.0	5.0	1.0
4. CH_3COOK	1.06	0.0	5.0	1.0
5. KCl	1.08	0.1	10.0	2.0
6. K_2HPO_4	1.44	0.1	10.0	2.0
7. $K_2B_4O_7.4\,H_2O$	1.05	0.0	5.0	2.0

Note: The main peak used was that at Ep_1 in all systems except that with K_2HPO_4 where the peak at Ep_3 was used.

3.3.2.1 The Effect of Accumulation Potential on Peak Height

The dependence of the adsorptive stripping peak current of norfloxacin on the accumulation potential was examined over the range of 0.0V to -0.8V. It is observed from Fig. 3.4 that the largest peak current was observed for accumulation potential in the range 0.00V to -0.10V for all the base electrolytes tested. The same information is reported for ciprofloxacin (27, 19); a compound from the same family of the 4-quinolones. The peak height started decreasing when the accumulation potentials became more negative than -0.1V. This phenomenon may be explained on the basis that norfloxacin species adsorb more strongly at potentials more positive than the electrocapillary maximum, at which potentials, the mercury surface is positively charged (42). As a result, a potential of 0.00V or -0.1V (depending on base electrolyte) was used through out this work (Table 3.2), since it provides the best signal-to-background characteristics.

3.3.2.2 Effect of Accumulation Time

The effect of prolonged accumulation times on norfloxacin adsorption was investigated in different base electrolytes as indicated in Table 3.2. The results are shown in Fig. 3.5. The longer the preconcentration time, the more analyte is adsorbed and the larger peak current was observed. A plateau was reached where the peak current ceased to increase with accumulation time. This could be explained in terms of saturation equilibrium if the system follows Langmuir isotherm where the mercury drop is totally covered by the adsorbates. It may be also explained in terms

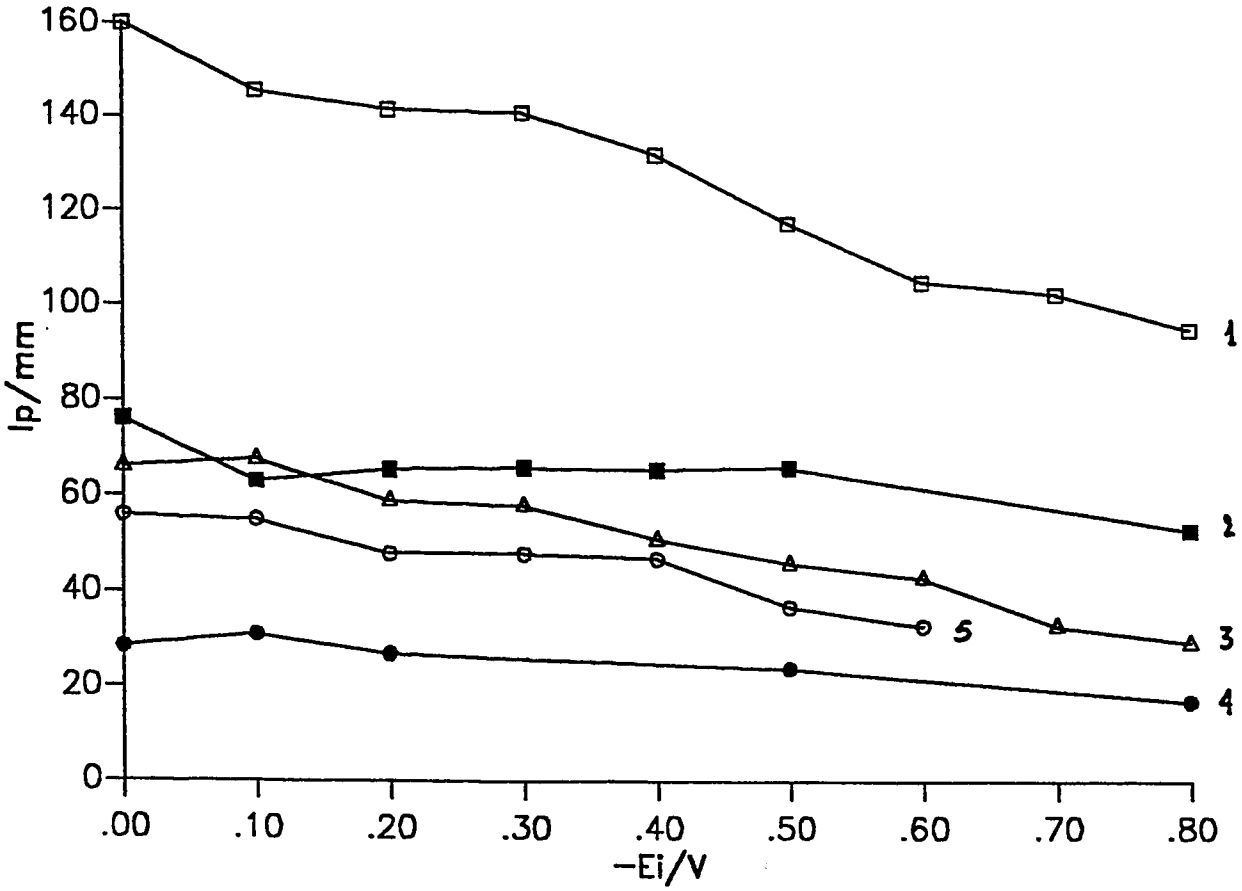


Figure 3.4 Effect of accumulation potential on norfloxacin peak height in 0.1M of different base electrolytes. (1) LiCl, (2) CH_3COOK, (3) NaOAc, (4) NaCl/NaOH and (5) KCl. Norfloxacin concentration was $1x10^{-6}$M. Scan rate used was 10mV/sec, accumulation time 1min and current sensitivity was 2μA.

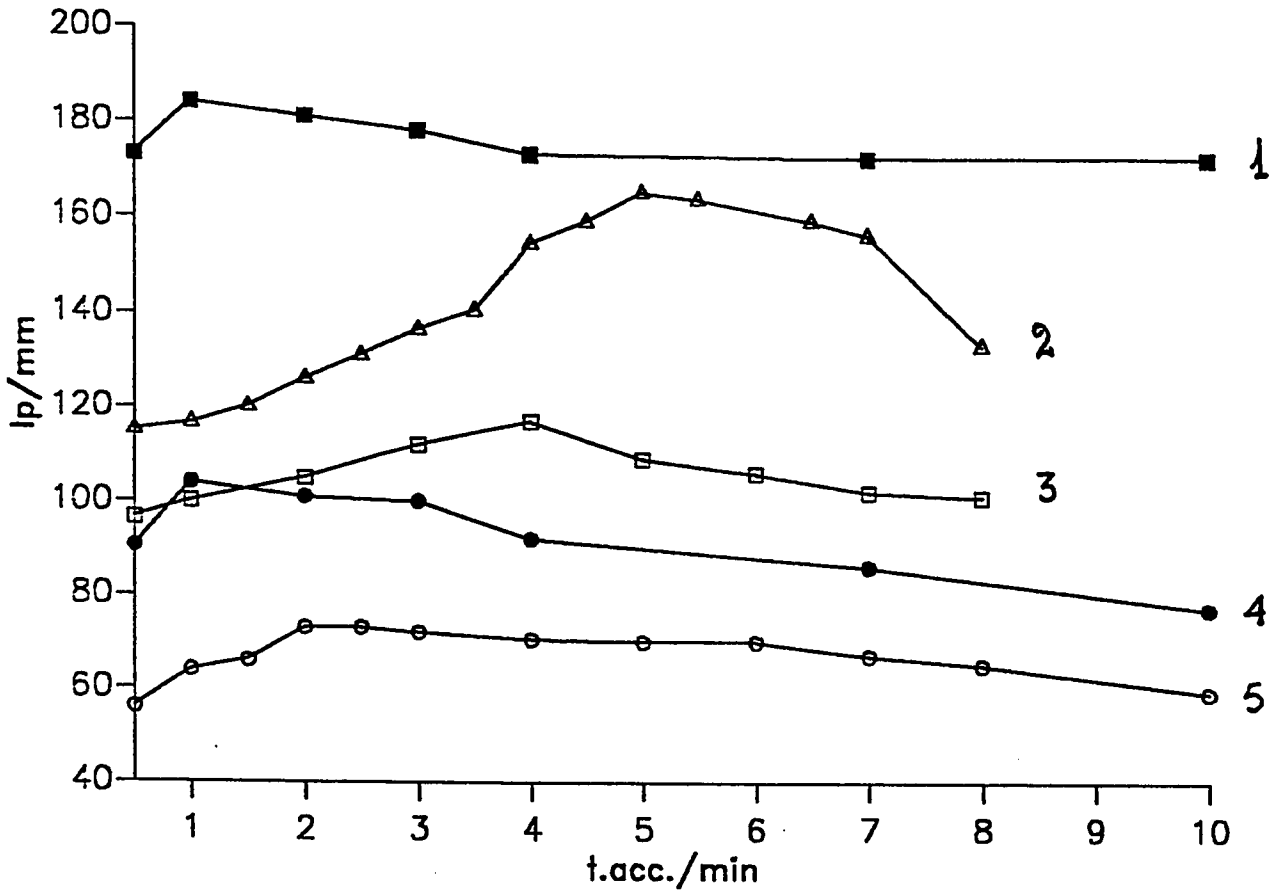


Figure 3.5 Effect of accumulation time on norfloxacin peak height in 0.1M of different base electrolytes. (1) CH_3COOK, (2) NaOAc, (3) LiCl, (4) NaCl/NaOH and (5) KCl. The norfloxacin concentration used was $1x10^{-6}M$, accumulation potential was OV and scan rate used was 10mV/sec.

of adsorption equilibrium, that is a function of the bulk concentration of the drug. This equilibrium may be reached as a compromise between the adsorption forces driving the molecules to the surface of the electrode and the possible repulsion interactions between the molecules in the adsorbed state and those in the bulk once a given coverage of the electrode has been reached (30).

3.3.2.3 Effect of Scan Rate

Another condition that affects the adsorptive response is the scan rate. Its effect was examined over a range of 2 mV/sec to 100 mV/sec with the aim of optimizing the practical conditions for a rapid and sensitive method of determination. The results are observed in Fig. 3.6, and the optimum scan rates used with different base electrolytes throughout the study are summarized in Table 3.2. These scan rates were used in all subsequent adsorptive measurements as these rates yielded optimum values with respect to both sensitivity and resolution.

3.4 Linearity of Calibration Plots for Norfloxacin in Various Base Electrolytes

The concentration dependance of the AdSV peak heights of norfloxacin in the presence of various base electrolytes was checked for the three well defined peaks at ~ -1.06V, ~ -1.28V and ~ -1.45V in solutions of pH ranges of slightly acidic to slightly alkaline. The linearity was tested for norfloxacin concentration range of 0.321 to 950 ppt (1.00×10^{-12} M to 2.98×10^{-9} M) and from 0.2570 to 65 ppb (8.05×10^{-10} M to 2.04×10^{-7} M). The results are shown in Table 3.3. Sometimes, more than one

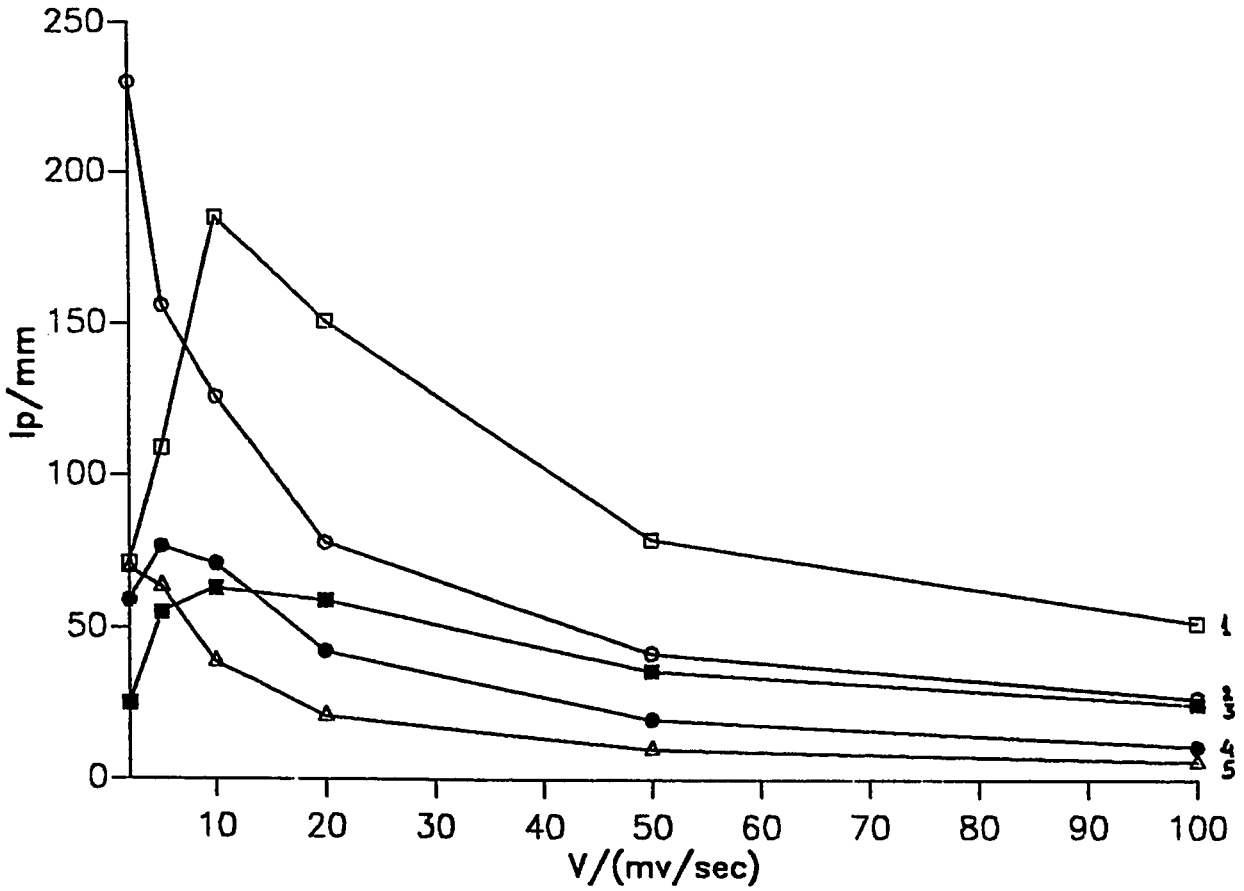


Figure 3.6 Effect of scan rate on norfloxacin peak height in 0.1M of different base electrolytes. (1) NaCl/NaOH, (2) LiCl, (3) KCl, (4) CH_3COOK and (5) $K_2B_4O_7$. Norfloxacin concentration used 1 x 10^{-6} M, accumulation time was 1min and accumulation potential OV.

Table 3.3: Linearity of calibration plots for norfloxacin in 0.1 M solutions of various base electrolytes. Using the three well defined peaks as observed from the table below current sensitivity was 1μA for ppt level and 2 μA for ppb level.

Base Electrolyte	- E_p range (V)	t_{acc} (sec)	Conc. range ppt or ppb	Corr. Coeff.	Slope (x 10^{-6}) μA.L.ng^{-1}
1. LiCl	1.05 / 1.18	20	0.32 - 250 ppt	0.9982	1.32
	1.02 / 1.04	240	0.25 - 25.0 ppb	0.9992	16.97
	1.26 / 1.28	240	0.32 - 3.0 ppt	0.9993	63.82
	1.27 / 1.30	20	80 - 200 ppt	0.9966	0.20
	1.40 / 1.48	240	2.0 - 5.0 ppb	0.9998	30.70
2. NaCl/NaOH pH = 8.80	1.06 / 1.07	60	3.23 - 3.5 ppb	0.9940	4.50
	1.21 / 1.23	60	0.63 - 5.0 ppt	0.9995	13.12
	1.45 / 1.53	60	0.63 - 6.0 ppt	0.9990	20.60
3. CH_3COONa	1.05 / 1.07	300	3.23 - 35 ppt	0.9952	5.17
	1.05 / 1.06	300	0.32 - 2.0 ppb	0.9980	10.99
4. CH_3COOK	1.07 / 1.18	20	50 - 400 ppt	0.9965	0.57
	1.03 / 1.07	60	0.32 - 60 ppb	0.9992	11.53
	1.28 / 1.32	60	0.63 - 8.0 ppt	0.9993	12.49
	1.30 / 1.32	20	550 - 850 ppt	0.9982	0.49
	1.44 / 1.50	60	0.63 - 7.0 ppt	0.9996	29.37
	1.48 / 1.54	20	300 - 950 ppt	0.9980	0.20

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Table 3.3 (Continued)

Base Electrolyte	- E_p range (V)	t_{acc} (sec)	Conc. range ppt or ppb	Corr. Coeff.	Slope (x 10^{-6}) $\mu A.L.ng^{-1}$
5. KCl	1.06 / 117	120	6.46 - 32 ppt	0.9981	1.80
	1.06 / 117	120	45 - 100 ppt	0.9955	0.35
	1.05 / 1.08	120	6.46 - 65 ppb	0.9990	5.44
	1.28 / 1.34	120	60 - 160 ppt	0.9982	0.22
	1.43 / 1.47	120	50 - 200 ppt	0.9902	0.087
	1.43 / 1.47	120	200 - 400 ppt	0.9992	0.28
	1.43 / 1.47	120	400 - 550 ppt	0.9999	0.083
6. K_2HPO_4	1.06 / 1.13	120	0.65 - 30 ppb	0.9963	1.48
	1.06 / 1.13	120	30 - 45 ppb	0.9990	1.39
	1.45 / 1.51	120	0.65 - 40 ppb	0.9967	4.66
7. $K_2B_4O_7.4H_2O$	EP2 + EP3 EP2 = -1.04V EP3 = -1.38V	120	25 - 60 ppb	0.9957	3.52

linear segment have been observed in Fig. 3.7. The breaks may be ascribed to a change in the mechanism of reduction at the electrode surface. It has been observed also that the AdSV peak potentials are concentration dependent. All of the three peaks showed a significant shift to more negative potential with increasing concentration.

The peak at -1.06 V was used for analysis since it is always the best well defined one and far from the discharge of the base electrolyte. It gave reasonable linearities within a rectilinear range from 0.3206 to 400 ppt (1.00×10^{-12} to 1.25×10^{-9} M) and from 0.2570 to 60 ppb (8.05×10^{-10} M to 1.88×10^{-7} M) norfloxacin. Slopes and correlation coefficients are shown in Table 3.3. Hence, this peak was the most extensively used to test the validity of the method for the determination of the drug in commercial tablets (Noroxin).

The coupling of adsorptive and catalytic effects, and the use of the optimum conditions obtained for the different methodological and instrumental variables for the accumulation of norfloxacin, resulted in extremely low detection limits (0.321 ppt or 1.00×10^{-12}M) following short accumulation periods. The shift of the AdSV peaks to more positive potentials (> 400 mv) comparing to the normal dpp observed previously, and the leveling of the current observed in Fig. 3.7 may be indicative of the catalytic behavior occurring at the surface of the electrode during the reduction process (8). The limiting value of the current reached with increasing concentration could be explained in terms of saturation equilibrium if the system follows the Langmuir isotherm, or, in terms of adsorption equilibrium which is a function of the bulk concentration of the drug, once a given coverage of the electrode has been reached as previously mentioned.

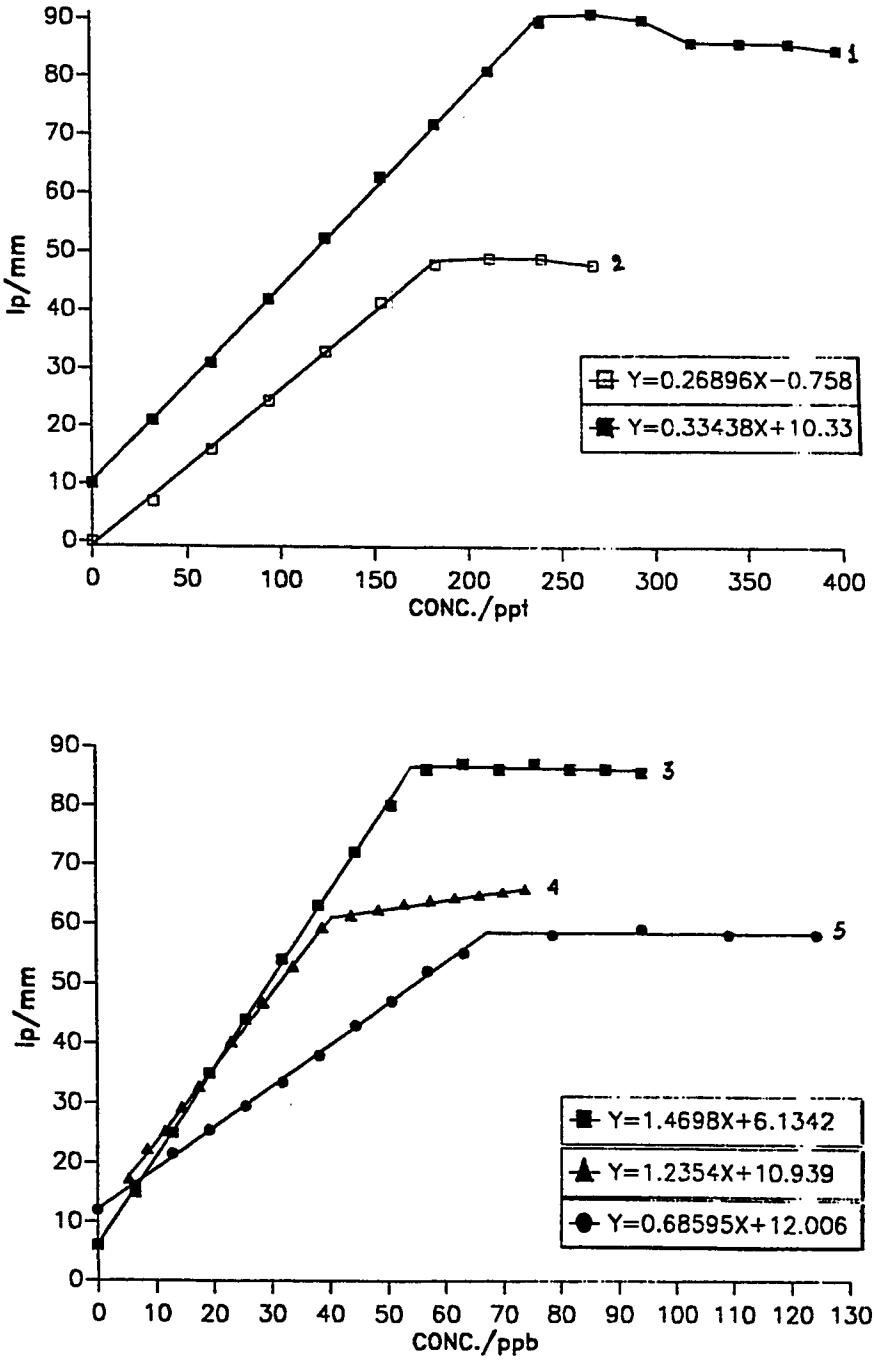


Figure 3.7 Calibration plots for norfloxacin under the optimum conditions of measurements (Table 3.2) in 0.1M of different base electrolytes. (1) LiCl, (2) NaOAc, (3) CH_3COOK, (4) K_2HPO_4 and (5) KCl. Current sensitivity used was 1uA for the ppt level and 2μA for the ppb level.

3.5 Analytical Application

Determination of Norfloxacin in Formulated Tablets (Noroxin) by AdSV

Norfloxacin was subsequently determined in Noroxin tablets by AdSV following the procedure indicated in the experimental section and employing the three reduction peaks mentioned above. The results obtained are summarized in Table 3.4. Incremental standard additions to different unknown samples of Noroxin resulted in well defined adsorptive stripping peaks (Fig. 3.8). The quantity of norfloxacin in the assayed samples was quantified based on the resulting standard addition plots (Fig. 3.9). Consecutive analysis yielded an average recovery of 110.77% and relative standard deviation of 8.25% for 14 unknown samples with different norfloxacin concentrations in 5 different base electrolytes as shown in Table 3.4.

This high recovery may be due to some interferences from adjacent peaks during the measurement of the peak heights for analysis. In most cases, the base electrolytes used showed small adsorptive peaks in the vicinity of the norfloxacin peaks at a potential starting at -1.02 V, thus obscuring the measurements eventhough after their subtraction from the total signal. Moreover, the drug excipients may adsorb and add to the background. Hence, careful studies should be made to verify the reasons for these high recoveries.

Table 3.4: Determination of norfloxacin in Noroxin tablets using the standard addition method. Accumulation time with stirring, initial potential and scan rate were as indicated in Table 3.2. current sensitivity 1μA for ppt level and 2μA for ppb level. The adsorption peak at -1.06V was used in all cases except in the case of NaOH/HCl where the peak at -1.28V was used.

Base Electrolyte	t_{acc} (sec)	range ppt or ppb	Correlation Coefficient	Quantity added ppt or ppb	Quantity found ppt or ppb	Recovery %
1. LiCl	240	0.32 - 3.0 ppt	0.9988	0.950	1.070	112.6
	20	0.32 - 140 ppt	0.9987	65.10	65.43	100.5
	20	0.32 - 160 ppt	0.9998	65.10	70.41	108.2
	20	0.32 - 250 ppt	0.9957	125.0	137.0	109.6
	240	0.25 - 16 ppb	0.9992	6.51	8.49	130.4
2. NaCl/NaOH pH = 8.80	60	0.63 - 5.0 ppt	0.9931	3.50	3.77	107.7
	60	0.63 - 5.0 ppt	0.9994	3.50	3.37	96.30
	60	3.23 - 30 ppb	0.9974	6.51	6.99	107.4
3. CH_3COONa	300	3.23 - 35 ppt	0.9990	6.51	7.87	120.9
	20	0.32 - 3.5 ppb	0.9985	1.31	1.59	121.3
	300	0.32 - 20 ppb	0.9952	13.10	13.66	104.3
4. CH_3COOK	60	0.32 - 2.0 ppb	0.9960	0.65	0.67	103.1
	60	0.32 - 35 ppb	0.9983	13.10	15.33	117.0
5. KCl	120	6.46 - 50 ppb	0.9971	13.10	14.50	110.7
					Average R.S.D.	110.7 8.25

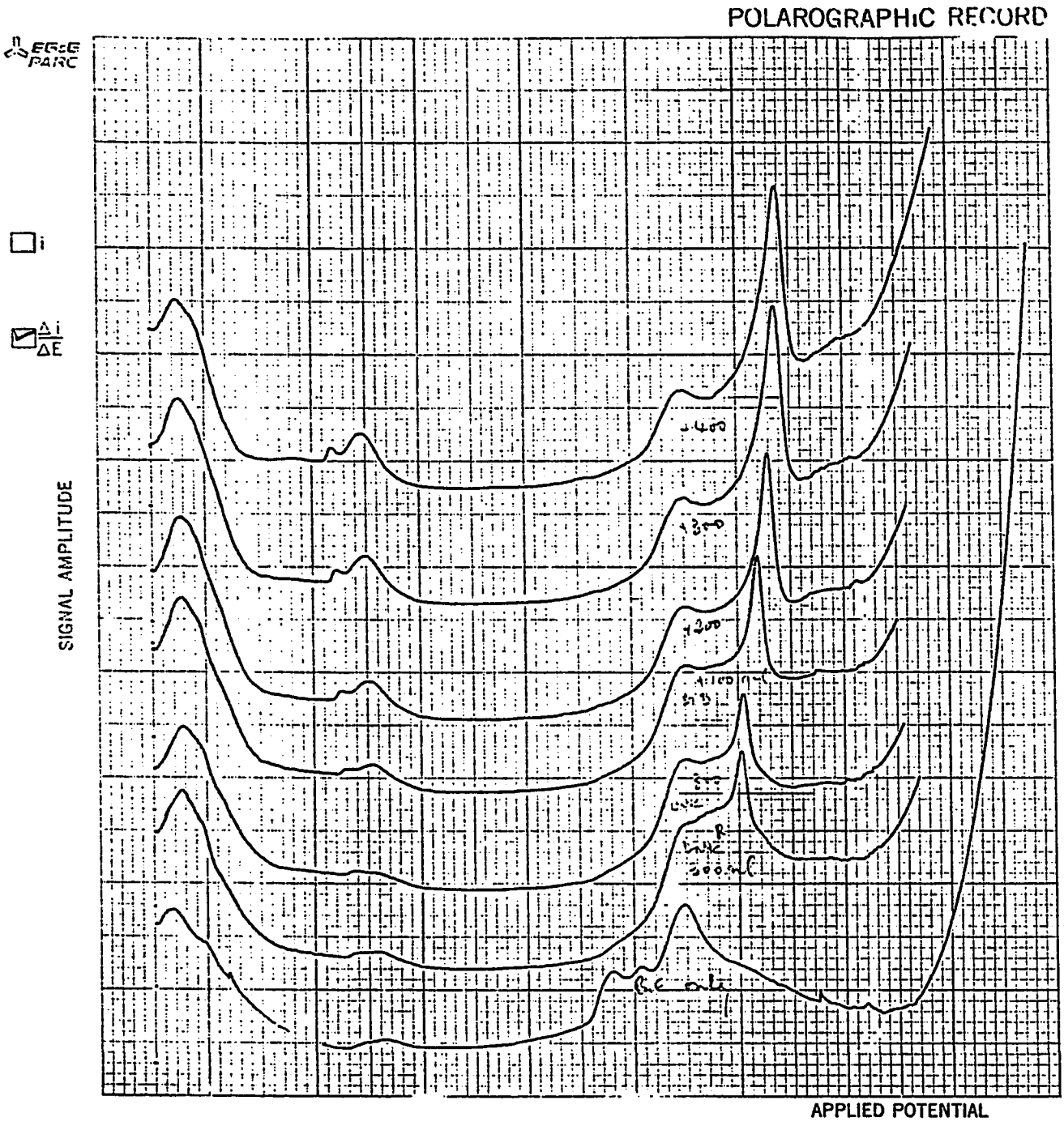


Figure 3.8 Dp adsorptive stripping voltammograms for the determination of norfloxacin in noroxin tablets by the standard addition method in 0.1M LiCl. The sensitivity used was 1μA.

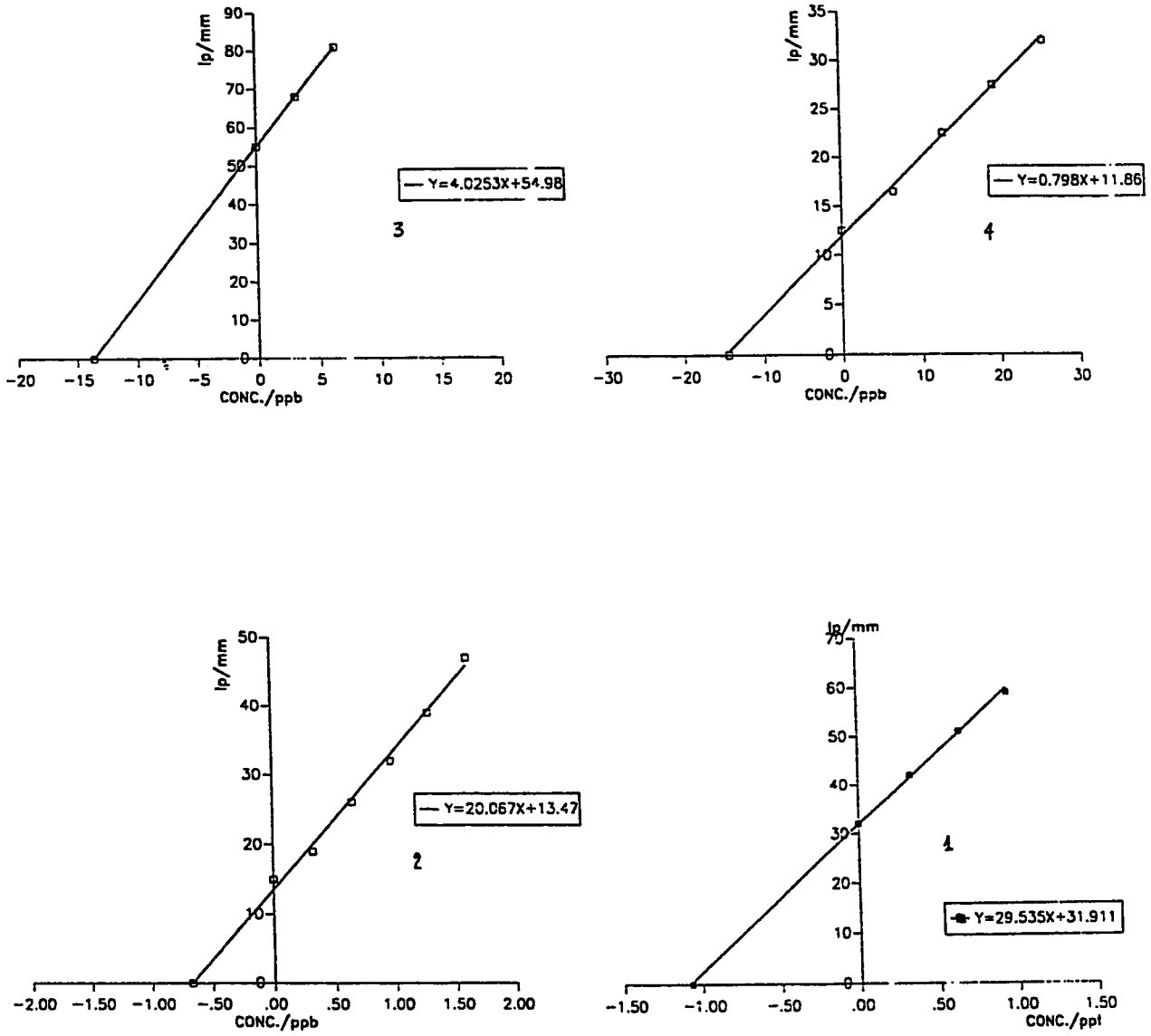


Figure 3.9 Standard addition plots for the determination of norfloxacin in Noroxin tablets in 0.1M of different base electrolytes. (1) LiCl, (2) CH_3COOK, (3) NaOAc and (4) KCl. Current sensitivity used was 1uA of ppt level and 2μA for ppb level.

CHAPTER FOUR

LINEAR SWEEP AND CYCLIC VOLTAMMETRIC BEHAVIOR OF NORFLOXACIN AND NALIDIXIC ACID

4.1 Introduction:

The first commercially available quinolone product was nalidixic acid (1-ethyl-1, 4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carboxylic acid). It is an antibacterial agent which has been used extensively in the treatment of gram-negative urinary tract infections. It is heat-stable but sensitive to photodecomposition, soluble in polar organic solvents, and sparingly soluble in water. The major metabolite of nalidixic acid is the 7-hydroxy methyl analogue (chapter two) which exhibits at least in vitro antibacterial activity nearly identical in spectrum potency to that of the parent compound (26). Recently chromatographic methods have been published for the determination of nalidixic acid and its active metabolite in biological fluids (26).

However, the breakthrough to finding broad spectrum quinolones was made in the early 1980's when fluorine was substituted at position 6, a piperazinyl residue was introduced at position 7 and new residues were introduced at position 1 of the quinolone basic structure (chapter 1). These structural modifications brought about a major improvement in

antibacterial activity against gram-negative bacteria, such as pseudomonas aeruginosa (which can cause lung infections) and to gram positive bacteria. The first commercially available products from this group of drugs (4-quinolones) for antibacterial chemotherapy where the fluoroquinolones, which bear very little resemblance to the nalidixic acid derivatives that did not contain fluorine. They were based on the active ingredients norfloxacin, enoxacin, or ofloxacin. They were soon followed by ciprofloxacin, which has become the reference substance for the modern fluoroquinolones.

In this work, the electrochemical techniques of potential sweep voltammetry and cyclic voltammetry at a hanging mercury drop electrode are utilized to investigate the electrochemical properties and assays of nalidixic acid and norfloxalin. The reduction patterns of norfloxacin and nalidixic acid in the presence of dimethyl formamide are further examined here in more details and applied for their determination in commercial tablets.

4.1.1 Linear Sweep Voltammetry and Mechanistic aspects of Electrochemical reactions

Linear sweep voltammetry (LSV) technique is based upon scanning the applied potential across the stationary electrode-solution interface linearly from an initial potential, E_i to a final potential E_f at a constant scan rate, V. The current is measured during the potential scan in an unstirred solution and recorded as a LS voltammogram. The voltammogram appears as a peak current with a subsequent decay due to depletion of electroactive species near the electrode surface.

Two useful parameters can be deduced from the LS voltammogram, namely, the peak potential, E_p and the peak current, i_p which correspond to the potetial and current at the voltammogram. For a reversible system, the peak current is given by the Randles-Sevcik, equation:

$$i_p = (2.69 \times 10^5)\, n^{3/2}\, A\, D_o^{1/2}\, V^{1/2}\, C_o$$

where A is the electrode surface area (cm^2), V is the scan rate (V/sec), C_o^* is the bulk concentration of the electroactive species (mol/l) and the other terms are as mentioned before.

However, the peak potential is defined by the equation,

$$E_p = E_{1/2} - 1.1\, RT/nF$$

where $-1.1\ (RT/nF) = -28.5 / n$ mV at 25°C

Sometimes, it is more convenient to report the potential as half-peak potential, $E_p/2$ which is given by the equation,

$$E_{p/2} = E_{1/2} + (1.1\, RT/nF) = E1/2 + 28.0/n \text{ mV at } 25^oC$$

or

$$E_p - E_{p/2} = (2.2\, RT/nF) = 56.5/n \text{ mV at } 25^oC$$

Thus, for a reversible wave, E_p is independent of scan rate and i_p (as well as the current at any other point on the voltammogram) is proportional to $V^{1/2}$ and a plot of i_p vs $V^{1/2}$ should be a straight line. A term called, the current function, which is $i_p / V^{1/2} C_o^*$ and depends on $n^{3/2}$ and $D^{1/2}$, can be used to estimate n for an electrode reaction, if a value of D_o can be estimated by the analogy between the electrochemical reactant and a compound of similar size or structure which undergoes an electrode

reaction with a known n value.

For a totally irreversible charge transfer (where the rate of electron transfer is sufficiently slow compared with V), the peak current is given by the equation,

$$(i_p)_{irr} = (2.99 \times 10^5)\, n\, (\alpha n_a)^{1/2}\, A\, C_o^{*}\, D_o^{1/2}\, V^{1/2}$$

where n_a is the number of electrons transferred up to and including the rate determining step.

The peak potential for a totally irreversible system, E_p, may be expressed by the following equations:

$$E_p = E_o' - \frac{RT}{\alpha n_a F}\, 0.780 + \ln \frac{(D_o)^{1/2}}{(K_o)} + \ln \frac{(\alpha n_a F v)^{1/2}}{(RT)^{1/2}}$$

where K_o is the rate of electron transfer.

$$E_p = E_{p/2} = \frac{1.857\, RT}{\alpha n_a F} = \frac{47.7}{\alpha n_a}\ \text{m V at } 25^{\circ}\text{C}$$

Thus, for a totally irreversible wave, i_p is also proportional to C_o^{*} and $V^{1/2}$, but E_p is dependent on α, k^o and V. This dependence can be used as criteria for reversibility. It could be deduced that E_p shifts (for an irreversible reduction process) in a negative direction by an amount of $1.15\ RT / \alpha\, naF$ (or $30 / \alpha\, na$ mV at 25°C) for each tenfold increase in V.

4.1.2 Cyclic Voltammetry and Mechanistic Aspects of Electrochemical reactions

Cyclic voltammetry (CV) is a very useful modern electroanalytical technique. Its versatility combined with the ease of measurements has resulted in extensive use of CV in the fields of inorganic chemistry, organic chemistry, and biochemistry. Cyclic voltammetry is often the first experiment to be performed in an electrochemical study of a compound on an electrode surface.

Cyclic voltammetry is based upon changing the potential of a small, stationary working electrode linearly with time and measuring the resulting current. The excitational signal is a linear potential scan with a triangular waveform as shown in Fig. 4.1a. This excitation signal sweeps the potential of the electrode between two values, called initial and final switching potentials Fig 4.1b. The effectiveness of CV results from its capability for rapidly observing the redox behavior over a wide potential range.

The important parameters of a cyclic voltamogram are the magnitudes of the anodic peak current (i_{pa}), cathodic peak current (i_{pc}), anodic peak potential (E_{pa}) and cathodic peak potential (E_{pc}). The cathodic half-peak potential ($E_p/2$) and the half wave potential ($E_{1/2}$) are also useful parameters.

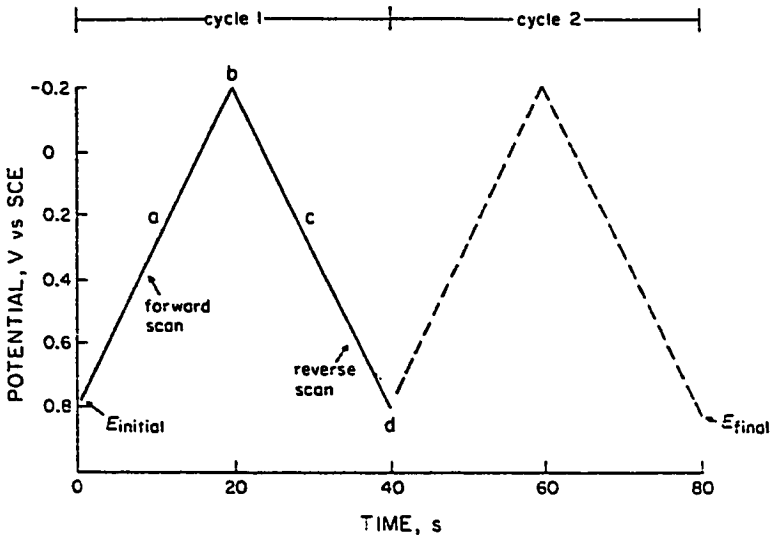


Figure 4.1a. Typical excitation signal for cyclic voltammetry—a triangular potential waveform with switching potentials at 0.8 and −0.2 V vs. SCE. *[Reprinted with permission from P. T. Kissinger and W. R. Heineman, J. Chem. Ed., 60, 702 (1983). Copyright © 1983, Division of Chemical Education, American Chemical Society.]*

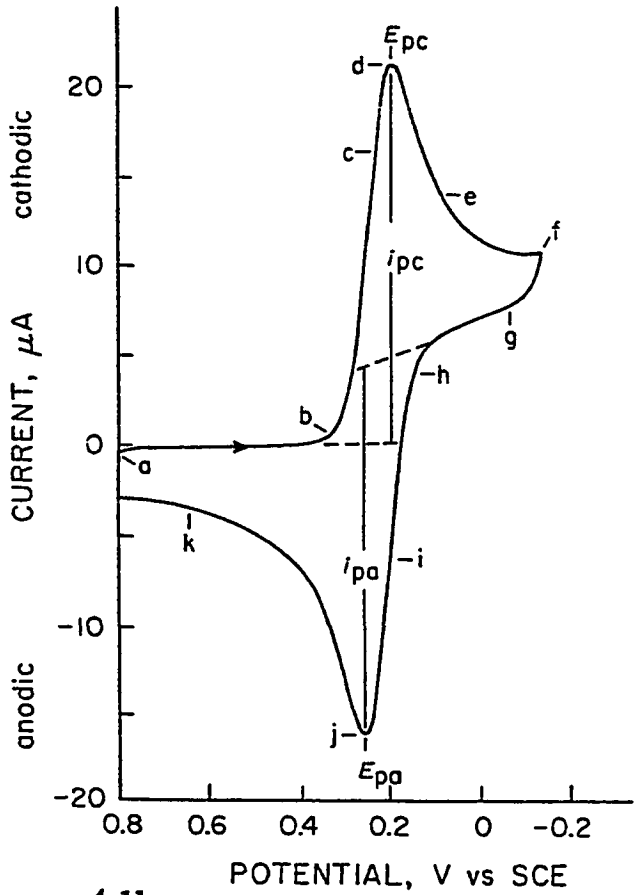


Figure 4.1b. Cyclic voltammogram of 6 m*M* $K_3Fe(CN)_6$ in 1 *M* KNO_3. Scan initiated at 0.8 V vs. SCE in negative direction at 50 mV/s. Platinum electrode area = 2.54 mm^2. *[Reprinted with permission from P. T. Kissinger and W. R. Heineman, J. Chem. Ed., 60, 702 (1983). Copyright © 1983, Division of Chemical Education, American Chemical Society.]*

The CV technique is useful in characterization of electrode reactions. The formal reduction potential ($E^{0'}$) for a reversible couple is centered between E_{pa} and E_{pc} (43, 14).

$$E^{0'} = \frac{E_{pa} + E_{pc}}{2}$$

The difference between the peak potentials can be used to determine the number of electrons transferred in the reversible electrode reaction as follows:

$$\Delta E_p = E_{pa} - E_{pc} = 2\,(1.1\,RT/nF) = \frac{0.059}{n}\ V \text{ at } 25^{o}C$$

This potential difference is independent of scan rate for the reversible couple, but slightly dependent on switching potential and cycle number (43). The values of i_{pa} and i_{pc} should be identical for a simple reversible couple of stable product, that is

$$\frac{i_{pa}}{i_{pc}} = 1$$

Electrochemical irreversibility is caused by slow electron exchange of the redox species with the working electrode. In this case equations for reversible cases are not applicable. Electrochemical irreversibility is characterized by a separation of peak potentials > 0.059/n (ΔEp increases with increasing scan rate V (3).

Many electrode reactions include purely chemical steps which take place in solution near the electrode and which can occur prior to electron transfer, following electron transfer or interposed between electron

transfer steps. Cyclic voltammetry is a powerful technique for detection and characterization of such coupled chemical reactions (3). When a chemical reaction follows the electrochemical reduction reaction, the process designated by EC reaction, where E signifies the electrochemical step and C the following chemical reaction. The C step can be first or second order, irreversible or reversible. More complex reactions are denoted by a string of letters in the order of the steps in the reaction scheme, e.g. CE, ECE, ECED, EEC. etc. Subscripts are sometimes included to denote reversibility, reaction order or other special features (4).

4.2 Experimental

4.2.1 Apparatus and Reagents

The linear sweep and the cyclic voltammetry techniques were performed with a PAR 264A voltammetric analyzer in conjunction with a PAR 303A static mercury drop electrode and an X-Y recorder (Model RE 0150) to record the voltammograms. A medium size hanging mercury drop electrode (HDME), with a 0.016 cm^2 surface area was employed as a working electrode. The reference electrode was Ag/AgCl electrode and a platinum wire served as the auxiliary electrode. The reagents used were from the same sources as mentioned in the previous chapters.

4.2.2 Analytical Procedures

10.0 ml of 0.1M of the base electrolyte was transferred to the cell. A stock solution of 0.01M norfloxacin or nalidixic acid prepared in dimethyl formamide was spiked into the base electrolyte. The solution was deaerated with nitrogen free oxygen for 8 min, and for 30 sec after each addition. However, in the case of the oxygen effect study the solution was not deaerated. A switching potential of 0V (unless stated otherwise) was applied to a fresh drop while the solution was unstirred. After 15 sec the voltammograms were recorded by applying a negative going linear sweep or cyclic scan with different scan rates mainly 50, 100 and 200 mV/sec, and a current sensitivity of 2μA. The scan was terminated after recording all of the peaks at -1.90 V.

In the linear scan voltammetric studies of norfloxacin and nalidixic acid, an aliquot of 10.0 ml of 0.1M of the base electrolyte was transferred to the cell and deaerated for 8 minutes. A stock solution of 0.01M norfloxacin or nalidixic acid prepared in DMF was spiked into the base electrolyte. A 30 sec deaeration period was applied after each addition and a 15 sec equilibration time was allowed. The linear scan current-voltage curves were then recorded using scan rates of 50 and 100 mV/sec and a current sensitivity of 2μA. The limiting currents were measured and calibration curves in several base electrolytes were constructed. The concentration ranges tested were from 10 ppm (3.13×10^{-5} M) to 300 ppm (9.40×10^{-4} M) for norfloxacin and from 10 ppm (4.30×10^{-5} M) to 215 ppm (9.5×10^{-4} M) for nalidixic acid. All data were obtained at room temperature.

4.2.3 Noroxin and Negram Sample Preparation

10 tablets of Noroxin or negram were ground to a fine powder. A quantity equivalent to one tablet was weighed, dissolved in DMF, transfered into a 100 ml volumetric flask and diluted to the mark with DMF. The solution was slightly turbid but no further treatment was made. Known volumes (0.05 to 1.00 mL) of the sample solution were added to 10 ml aliquots of the base electrolyte. Calibration solutions were prepared in the same manner. Generally 10 spikes were added to the base electrolyte for the purpose of calibration curves construction.

4.3 Cyclic Voltammetry Studies

Various buffered and unbuffered electrolytic solutions of 0.1M concentrations, namely, NaOAc, K_3 Citrate, $K_2B_4O_7.4H_2O$, K_2HPO_4, $(CH_3)_4NBr$, KCl, LiCl and HCl (2M) have been utilized as base electrolytes. The cyclic voltammetry data for 1 x 10^{-4} M norfloxacin and nalidixic acid in 1% DMF are reported in Tables 4.1 and 4.2 and Tables 4.3 and 4.4 respectively. Norfloxacin showed up to seven cathodic peaks (A-G) in slightly acidic to slightly alkaline media as observed from the cyclic voltammograms shown in Fig. 4.2. One main well defined wave in the range of -1.48 to -1.63V Wave F has been obtained in all base electrolytes used. The same behavior was reported for ciprofloxacin (19) and pipemedic acid (30), which belong to the same quinolones family.

However, nalidixic acid showed six cathodic peaks in supporting electrolytes o f slightly acidic to slightly alkaline media as observed from

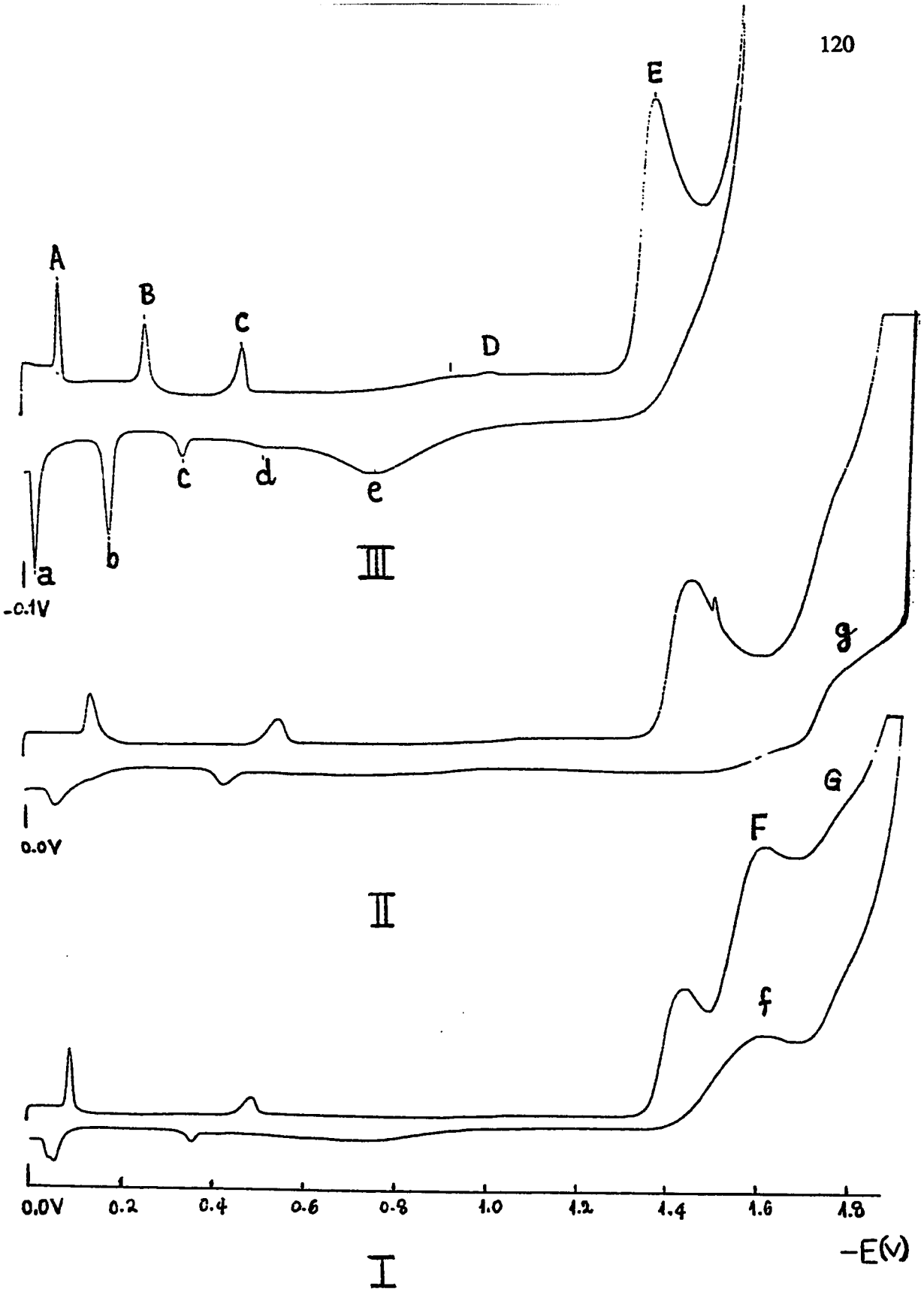


Fig. 4.2 Cyclic voltammograms for 1×10^{-4}M norfloxacin in 0.1M of different base electrolytes. (I) CH_3COONa / CH_3COOH, pH 7.0 (II) NaOAc, pH 8.0 (III) NH_3 / NH_4Cl, pH 8.5. The scan rate used was 50 mV/sec, and current sensitivity was 2 μA.

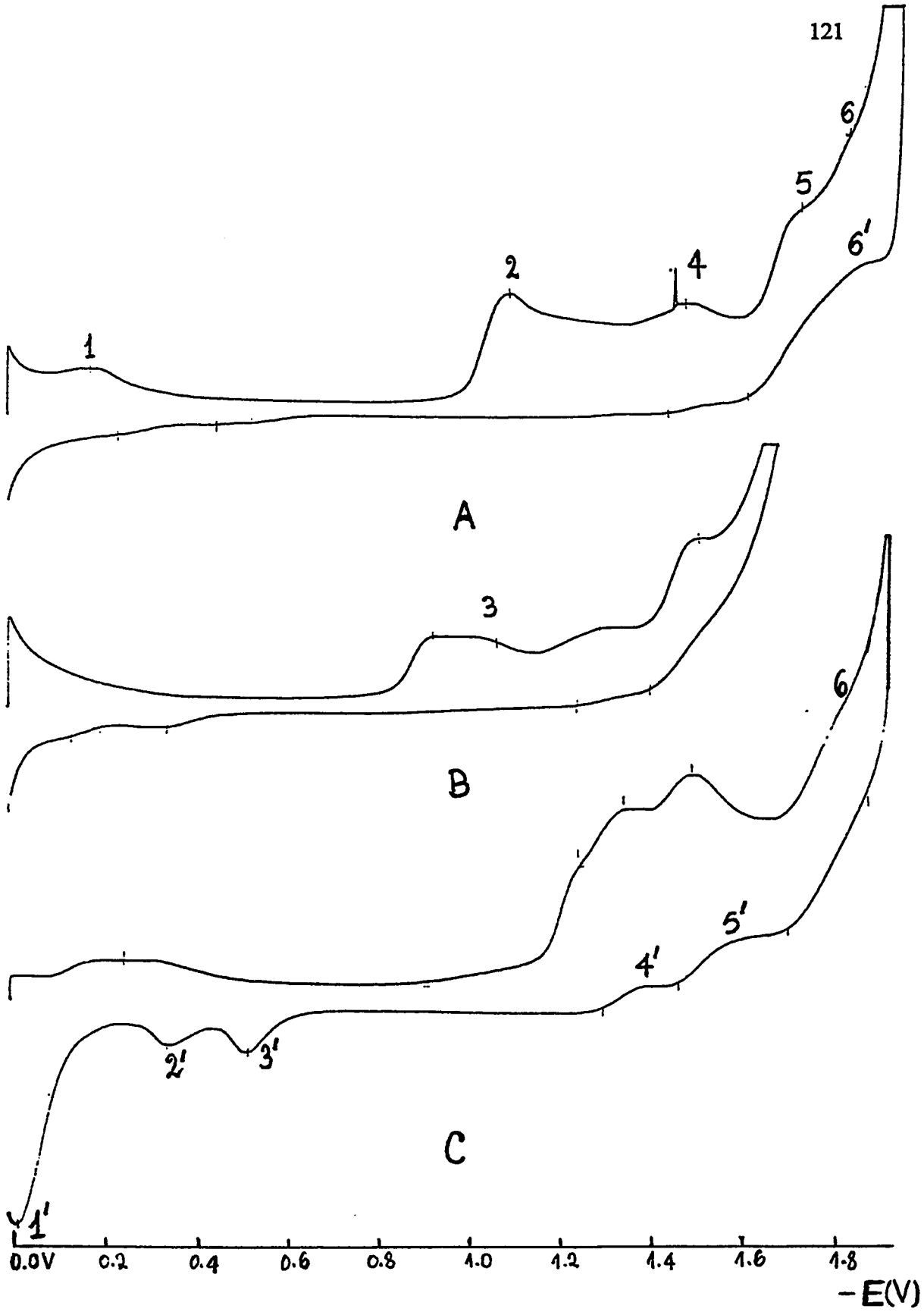


Fig. 4.3 Cyclic voltammograms for 1×10^{-4}M nalidixic acid in 0.1M of different base electrolytes. (A) KCl (B) $(CH_3)_4NBr$ (C) CH_3COONa / CH_3COOH (pH 7.5). The scan rate used was 50 mV/sec, and current sensitivity was 2 μA.

Table 4.1: Cyclic Voltammetric peak positions for norfloxacin appeared in the cathodic scan in different base electrolytes.

Base Elect. / Scan rate (mV/sec)	$-E_{P_A}^{C}$ (V)	$-E_{P_B}^{C}$ (V)	$-E_{P_C}^{C}$ (V)	$-E_{P_D}^{C}$ (V)	$-E_{P_E}^{C}$ (V)	$-E_{P_F}^{C}$ (V)	$-E_{P_G}^{C}$ (V)
NaOAc							
50	0.14	---	---	0.56	---	1.48	1.80
100	0.15	---	---	0.57	1.09	1.49	1.80
200	0.15	---	---	0.58	1.10	1.49	---
K_3 Citrate							
50	---	0.22	---	0.60	---	1.54	1.80
100	---	0.23	---	0.61	1.15	1.54	1.80
200	---	0.24	---	0.62	1.16	1.54	1.80
$K_2B_4O_7$							
50	---	0.22	---	---	1.24	1.59	1.80
100	---	0.22	---	---	1.24	1.60	1.80
200	---	0.24	---	---	1.25	1.61	1.80
K_2HPO_4							
50	---	---	---	---	---	1.62	1.80
100	---	---	---	---	1.18	1.62	1.80
200	---	---	---	---	1.18	1.63	1.81
$(CH_3)_4$ NBr							
50	---	0.28	0.44	0.49	1.20	1.56	1.77
100	---	0.29	0.44	0.48	1.20	1.56	1.78
200	---	0.29	0.45	0.48	1.21	1.56	1.78
KCl							
50	0.10	0.26	0.35	0.55	---	1.53	1.80
100	0.10	0.26	0.36	0.55	---	1.51	1.80
200	0.11	0.26	0.37	0.56	1.22	1.50	1.81
LiCl							
50	0.14	---	0.35	0.43	1.22	1.56	1.80
100	0.14	---	0.35	0.44	1.22	1.56	---
200	0.15	---	0.36	0.47	1.22	1.57	---
HCl (2M)							
50	---	---	---	---	1.02	---	---
100	---	---	---	---	1.02	---	---
200	---	---	---	---	---	---	---

Table 4.2: Cyclic Voltammetric peak positions for norfloxacin appeared in the anodic scan in different base electrolytes.

Base Elect. / Scan rate (mV/sec)	$-E_{Pa}^{a}$ (V)	$-E_{Pb}^{a}$ (V)	$-E_{Pc}^{a}$ (V)	$-E_{Pd}^{a}$ (V)	$-E_{Pe}^{a}$ (V)	Catalytic $-E_{Pf}^{a*}$ (V)	Waves $-E_{Pg}^{a*}$ (V)
NaOAc							
50	0.08	---	0.45	---	--	-1.58 / -1.68	1.80
100	0.08	---	0.45	---	0.80	---	1.80
200	0.09	---	0.46	---	0.86	---	---
K_3 Citrate							
50	---	0.18	0.51	---	0.86	1.62	1.80
100	---	0.18	0.50	---	0.85	---	1.80
200	---	0.18	0.49	---	0.87	---	1.80
$K_2B_4O_7$							
50	---	---	---	---	0.86	---	1.80
100	---	---	---	---	0.86	---	1.80
200	---	---	---	---	0.86	---	1.80
K_2HPO_4							
50	---	---	---	---	0.90	1.66	1.80
100	---	---	---	---	0.88	---	1.80
200	---	---	---	---	0.88	---	1.80
$(CH_3)_4$ NBr							
50	---	0.23	0.37	---	---	-1.50 / -1.55	-1.76
100	---	0.22	0.37	---	---	---	-1.75
200	---	0.22	0.39	0.42	0.86	---	-1.75
KCl							
50	0.06	0.27	---	0.47	0.86	1.32	-1.80
100	0.06	0.27	---	0.48	0.86	---	-1.80
200	0.06	0.26	---	0.50	0.87	---	-1.80
LiCl							
50	0.09	0.28	---	---	---	1.43 / 1.45	1.80
100	0.09	0.28	---	---	1.0	---	---
200	0.10	0.28	---	---	0.93	---	---
HCl (2M)							
50	---	---	---	---	---	---	---
100	---	---	---	---	---	---	---
200	---	---	---	---	---	---	---

* These waves are of catalytic nature.

Table 4.3: Cyclic Voltammetric peak positions for nalidixic acid appeared in the cathodic scan in different base electrolytes.

Base Elect. and Scan rate (mV/sec)	$-E_{P_1}^{c}$	$-E_{P_2}^{c}$	$-E_{P_3}^{c}$	$-E_{P_4}^{c}$	$-E_{P_5}^{c}$	$-E_{P_6}^{c}$
NaOAc						
50	0.14	---	1.36	1.52	1.78	---
100	0.16	---	1.35	1.49	1.78	---
200	0.18	---	1.35	1.52	1.79	---
K_3 Citrate						
50	0.16	1.08	1.36	1.54	1.76	---
100	0.18	1.10	1.36	1.56	1.76	---
200	0.18	1.14	1.36	1.60	1.76	---
$K_2B_4O_7$						
50	0.16	1.18	---	1.60	1.74	---
100	0.17	1.16	---	1.61	1.75	---
200	0.18	1.17	---	1.61	1.75	---
K_2HPO_4						
50	0.18	1.20	---	1.59	1.76	---
100	0.18	1.22	---	1.60	1.76	---
200	0.20	1.24	---	1.60	1.77	---
$(CH_3)_4$ NBr						
50	---	1.14	1.28	1.51	1.73	---
100	---	1.14	1.24	1.51	1.73	---
200	---	1.15	1.27	1.50	1.73	---
KCl						
50	0.18	1.11	---	1.50	1.76	1.86
100	0.19	1.12	1.30	1.50	1.76	---
200	0.20	1.13	1.32	1.47	1.77	---
LiCl						
50	0.20	1.10	---	1.52	1.72	1.86
100	0.20	1.11	---	1.50	1.72	1.88
200	0.22	1.13	1.33	1.50	1.74	1.90
HCl (1M)						
50	---	0.83	1.05	---	---	---
100	---	0.81	1.06	---	---	---
200	---	0.78	1.09	---	---	---

Table 4.4: Cyclic Voltammetric peak positions for nalidixic acid appeared in the anodic scan in different base electrolytes.

Base Elect. and Scan rate (mV/sec)	$-E_{P_1}^{a}$ (V)	$-E_{P_2}^{a}$ (V)	$-E_{P_3}^{a}$ (V)	$-E_{P_4}^{a}$ (V)	$-E_{P_5}^{a}$ (V)	$-E_{P_6}^{a}$ (V)
NaOAc						
50	0.03	0.39	0.55	1.36	1.58	1.86
100	0.02	0.37	0.54	1.36	1.58	1.86
200	0.01	0.34	0.50	1.36	1.58	1.84
K_3 Citrate						
50	0.08	0.48	0.60	---	1.60	1.90
100	0.07	0.48	0.60	---	1.60	---
200	0.05	0.43	0.56	---	1.60	---
$K_3B_4O_7$						
50	0.19	---	---	---	1.60	1.88
100	0.17	0.54	0.60	1.28	1.60	1.88
200	0.16	0.52	0.60	---	1.60	1.88
K_2HPO_4						
50	0.17	---	---	---	1.60	1.90
100	0.16	0.52	0.61	---	1.60	1.90
200	0.15	0.50	0.60	---	1.60	1.90
$(CH_3)_4$ NBr						
50	0.34	0.55	---	---	1.56	1.74
100	0.28	0.49	---	---	1.56	1.74
200	---	0.47	---	---	1.56	1.74
KCl						
50	---	---	1.20 *	1.16	1.56	1.90
100	0.22	0.48	---	---	1.56	1.90
200	0.20	0.46	---	---	1.56	1.90
LiCl						
50	0.23	0.42	1.20/1.23*	1.20	1.55	1.74
100	0.24	0.44	1.26	1.26	1.55	1.74
200	0.19	0.43	---	---	1.55	1.74
HCl (1M)						
50	0.23	---	---	---	---	---
100	0.23	---	---	---	---	---
200	0.21	---	---	---	---	---

* These waves are of catalytic nature

the cyclic voltammogram shown in Fig. 4.3. Three main well defined waves in the ranges of -1.24V to -1.36V (Wave 2), -1.47V to -1.61V (Wave 4) and from -1.72V to -1.79V (Wave 5) have been observed in each of the base electrolytes used.

In a medium of 2M hydrochloric acid norfloxacin exhibited only one wave at -1.02V which resembles a shoulder and did not show any shift with scan rate (Fig. 4.4 voltammogram 1). This wave started to disappear at scan rates > 50mV/sec and concentrations > $3x10^{-4}$M by taking a flatter shape until it joined the base electrolyte discharge curve. This wave has been found to be of low sensitivity to concentration with the linear scan voltammetry technique. Hence, it is not recommended to be used for analytical purposes with this technique.

Nalidixic acid gave rise to two irreversible waves in HCl (1M), (Fig. 4.4, voltamogram 2). The main wave is observed at -0.81V. It showed a cathodic shift with concentration and with scan rates $\geq$ 500mV/sec. A second small wave was observed at -1.06V. It didn't show any shift with concentration. However, a small cathodic shift was noted with scan rate. Both waves showed good sensitivity to concentration.

In the backward anodicscan, norfloxacin showed two cathodic waves (Fig. 4.5)which are indicative of a catalytic process (38) and this type of waves are known as catalytic waves (6). The main one appeared at a more positive potential with respect to the other one. It has been observed in the range of potential -1.32V to -1.68V when the scan rate was 50mV/sec but

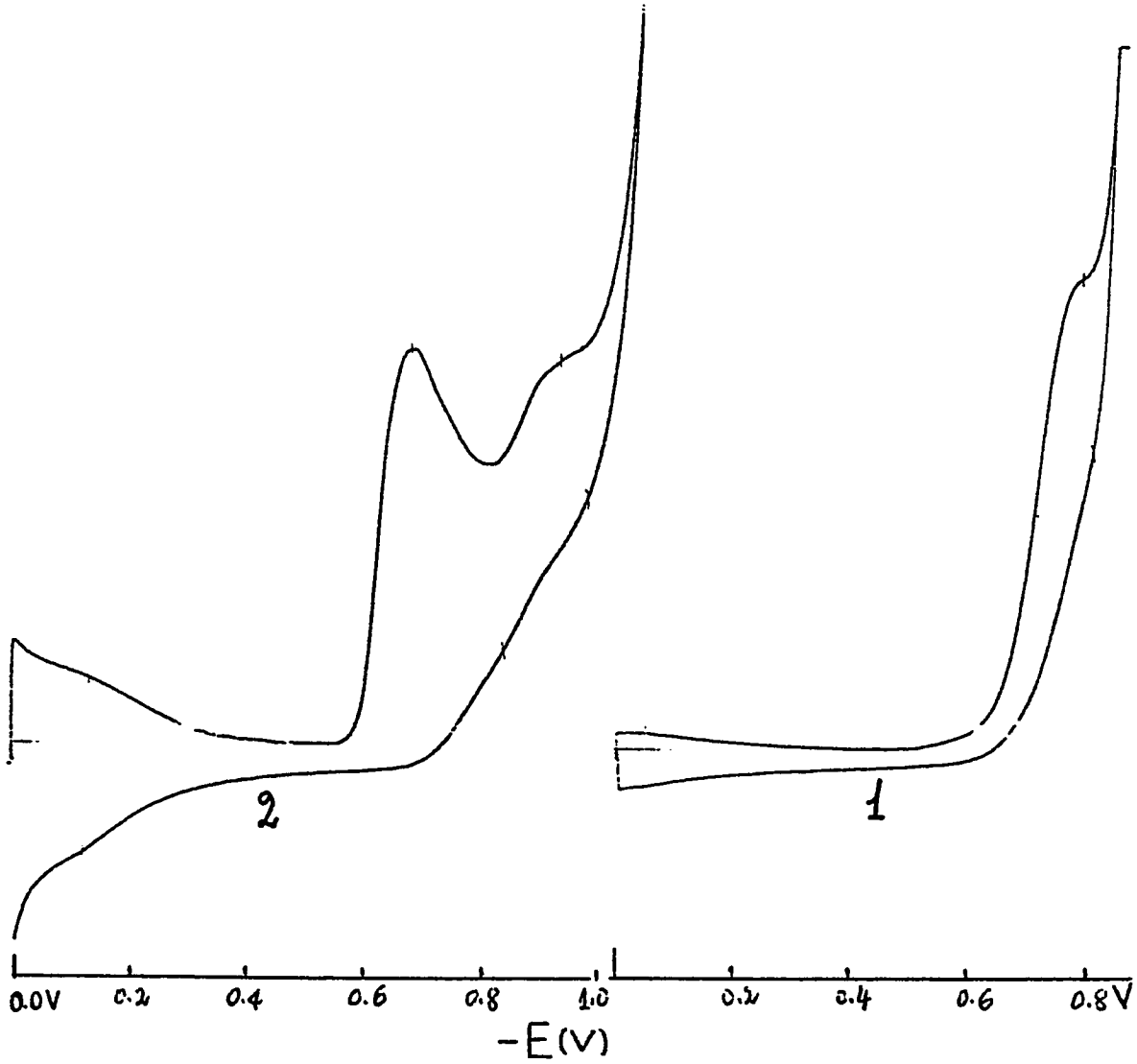


Fig. 4.4 Cyclic voltammograms for (1) 1×10^{-4}M norfloxacin in 2M HCl and (2) 1×10^{-4}M nalidixic acid in 1M HCl base electrolytes. The current sensitivity was 2 μA. The scan rate used was 50 mV/sec for norfloxacin and 100 mV/sec for nalidixic acid.

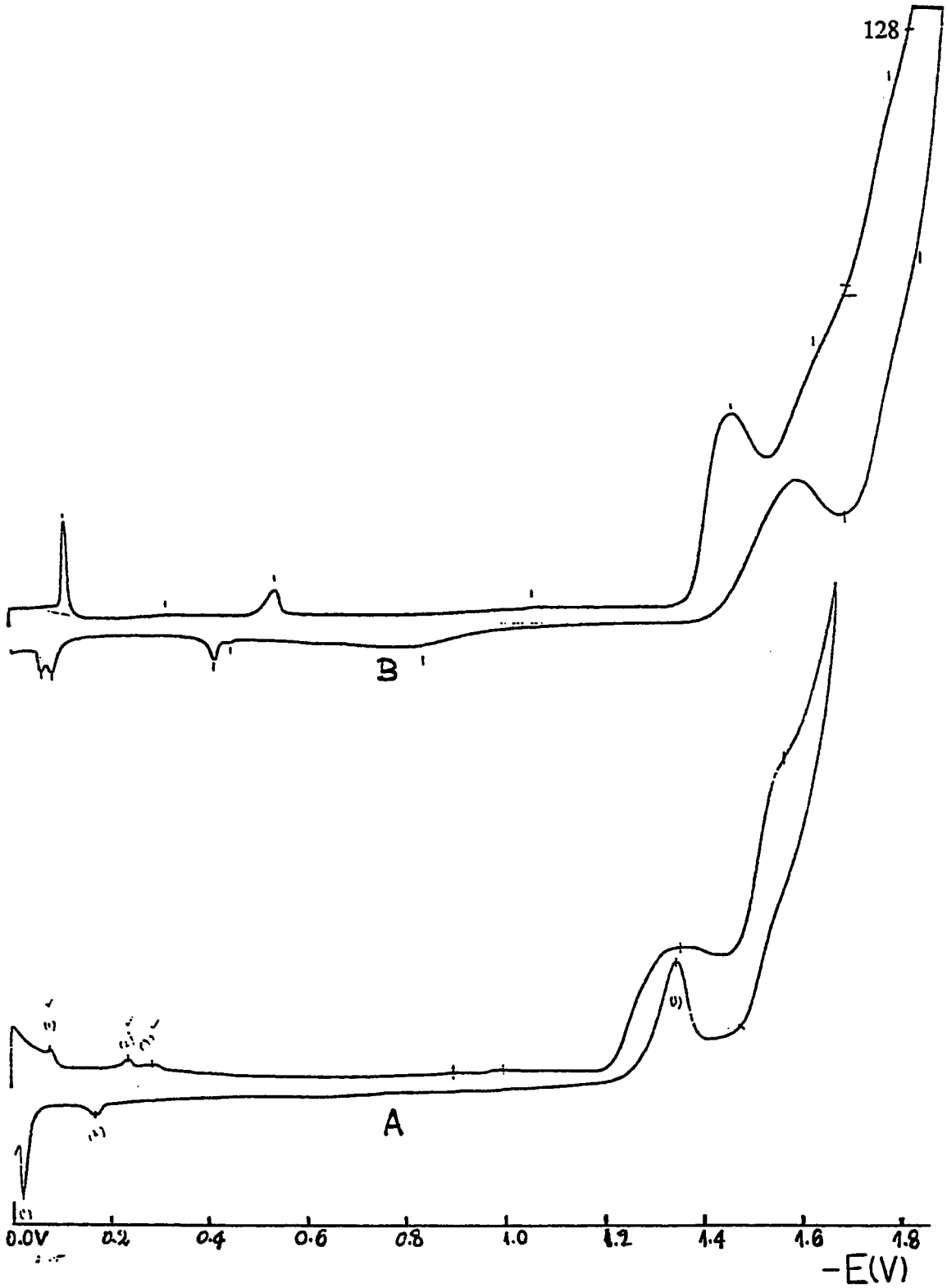


Fig. 4.5 Cyclic voltammograms for 1×10^{-4}M norfloxacin in 0.1M of different base electrolytes showing the catalytic waves obtained in the anodic scan. (A) $(CH_3)_4NBr$ and (B) CH_3COONa / CH_3COOH (pH 7.5). The scan rate used was 50 mV/sec and the current sensitivity was 2 μA.

did not appear at faster scan rates (Table 4.2). This behavior may be attributed to the slow formation of the catalyzed species. This catalytic wave was observed in almost all base electrolytes mentioned above and its position was dependant on the base electrolyte used as shown in Table 4.2. The other catalytic wave was less significant and it was also observed in all base electrolytes and at the various scan rates (50, 100 and 200 mV/sec) used in the range of -1.75V to -1.80V.

Nalidixic acid also showed two catalytic waves (Fig. 4.6). The first one appeared at a more positive potential and it is also the main catalytic wave as in the case of norfloxacin. It appeared only in two base electrolytes, namely KCl and LiCl, in the range of -1.70V to -1.76V when the scan rate were 50mV/sec and 100mV/sec, but it disappeared when the scan rate became 200 mV/sec. The other wave was again insignificant and was observed only in sodium acetate base electrolyte at a fixed potential of -1.85V with different scan rates 50, 100 and 200 mV/sec. These catalytic phenomena observed during the electroreduction process of nalidixic acid and norfloxacin may be responsible for the irreversibility of their main electrode processes (main waves) in the different base electrolytes used.

4.3.1 Effect of pH on the Cyclic Voltammetry Behavior of Norfloxacin

Cyclic voltammograms obtained for 4.0 x 10^{-4} M solutions of norfloxacin of different pH values (HCl, NaOAc/CH_3COOH, NH_3/NH_4Cl and NaOH) showed behavior resemble that observed the previous studies carried out using dc polarography and dp polarography mentioned in

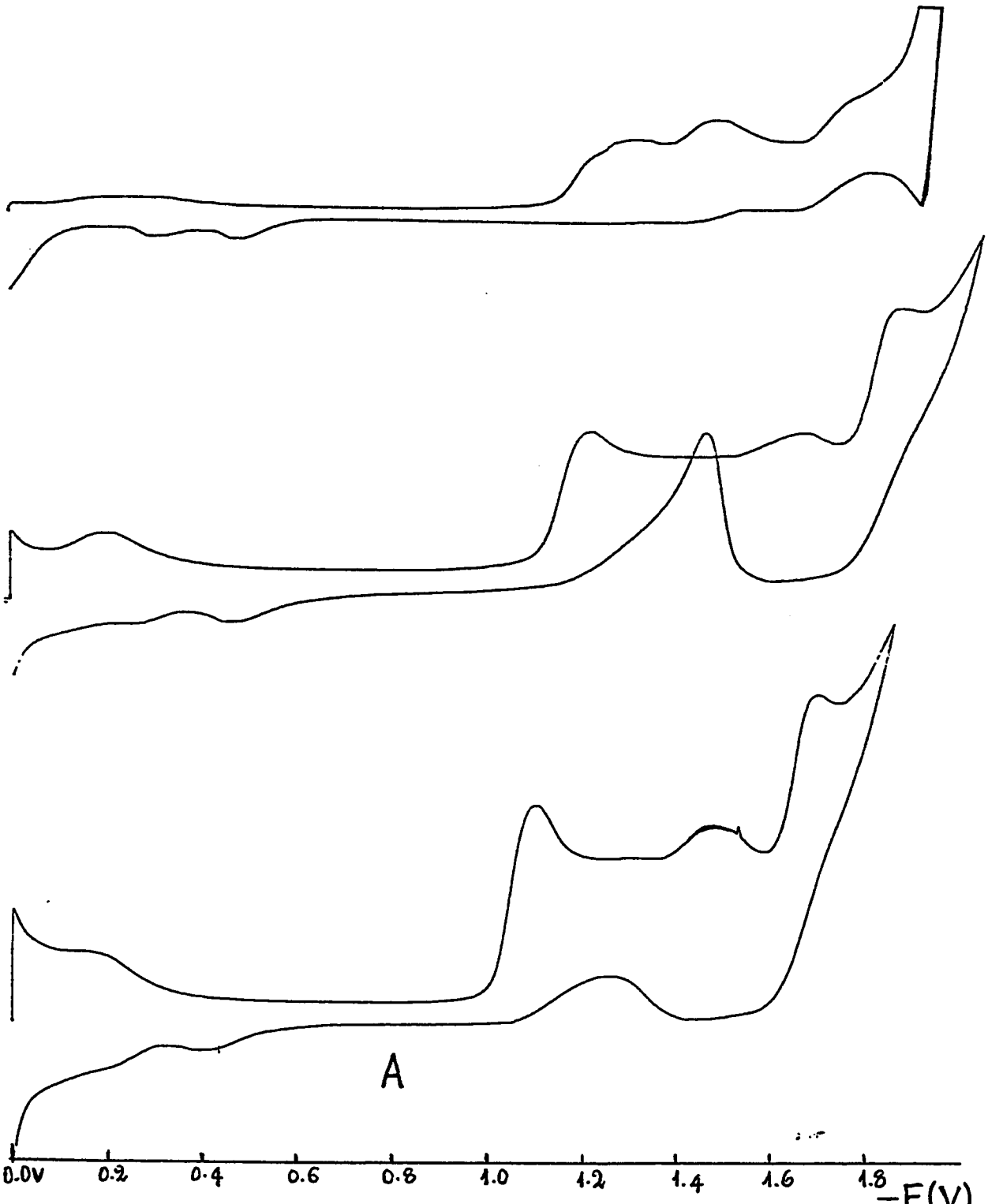


Fig. 4.6 Cyclic voltammograms for 1×10^{-4}M nalidixic acid showing the catalytic waves in the anodic scan, in 0.1M of different base electrolytes. (A) LiCl, scan rate used was 100 mV/sec (B) LiCl, 50 mV/sec (C) NaOAc, 200 mV/sec. In (A) and (B) current sensitivity was 2 μA and in (C) was 10 μA.

chapter two. Norfloxacin showed only one reduction wave at a potential in the range -1.0V to -1.02V in 2M and 1M HCl base electrolyte. The wave has diminished in height and became a shoulder in 1M HCl and finally disappeared in a solution of 0.1M HCl. However, with the dpp technique the wave disappeared in a solution of 0.01M HCl. When the pH was raised to higher values, the above mentioned reduction wave was replaced by seven waves scattered in the range -0.10V to -1.81V appearing at different pH values. In the pH range of 5.5 to 6.0, norfloxacin showed only one wave (wave E) in the potential of range -1.40V to -1.44V which characterises the main reduction process (main wave) of norfloxacin (wave D in chapter two). At pH 6.5 three other waves A, C and D, appeared in the potential ranges of -0.080V to -0.095V, -0.390V to -0.40V and -1.0V to -1.04V. Waves A and C are small and sharp. They resemble the two ill defined waves mentioned in the dpp polarograms. Wave D was very small and was observed only at scan rates $\geq$ 200 mV/sec. When the pH of the solution reached 7.0, two new waves F and G appeared in the ranges -1.60V to -1.68V and -1.80V to -1.82V respectively. In the anodic scan a huge catalytic wave was repeatedly observed in the potential range -1.60V to -1.66V at scan rates of 50, 100 and 200 mV/sec (Fig. 4.6, Wave F). The height of the wave was observed to be inversely proportional to the scan rate. At pH 7.5, the catalytic wave decreased in height and showed a small anodic shift. It was easily observed with a scan rate of 50 mV/sec but insignificant with 100 mV/sec, and disappeared with 200 mV/sec. When the pH was increased to 8.0, wave F disappeared and the wave at the most negative potential (wave G) started to disappear as well. At this pH, the main wave E reached a minimum peak height (v = 50mv/sec) and its

I_p - pH curve (Fig. 4.7) showed a break point by changing its trend from a negative slope to a positive slope. This break or change in behavior may be ascribed to a change in the mechanism of the electroreduction reaction at the surface of the electrode. This pH may correspond to the polarographic pK for norfloxacin as suggested in chapter two. At pH 8.5 another small and sharp peak (Wave B) appeared between waves A and C at a potential in the range of -0.37V to -0.38V. This wave also showed a sluggish reversibility due to its relatively large ΔEp (80 mV), and it appeared only at pH 8.5. At this pH, wave G disappeared completely and the I_p - pH curve for the main peak (wave E) resumed its decreasing behavior (Fig. 4.7). When pH became 9.0, waves A and C also disappeared and when it reached 9.5, wave G appeared again and its I_p - pH curve changed its trend from negative slope to positive slope (Fig. 4.7). This break and change in behavior is also ascribed to a change in the mechanism of the electroreduction reaction at the surface of the electrode concerning the second main peak (wave G) which is equivalent to wave D in the case of dpp in chapter two. This pH may correspond to the second polarographic pK for norfloxacin. At pH 10.5, the main peak disappeared completely, and only two peaks remained, at pH 11, (wave D) and wave G. When 0.1M sodium hydroxide was used as the base electrolyte, all waves vanished completely.

Fig. 4.8 shows the shift in the CV peak potentials for the peaks exhibited by norfloxacin with pH. E_p-pH dependance for wave E is described by two segments of E_p/pH of 43.77 mV and 113.6 mV below and above pH 8.5 respectively. E_p/pH for wave G was found to be -14.3 mV and 26 mV below and above pH 9.5, respectively. Other members of

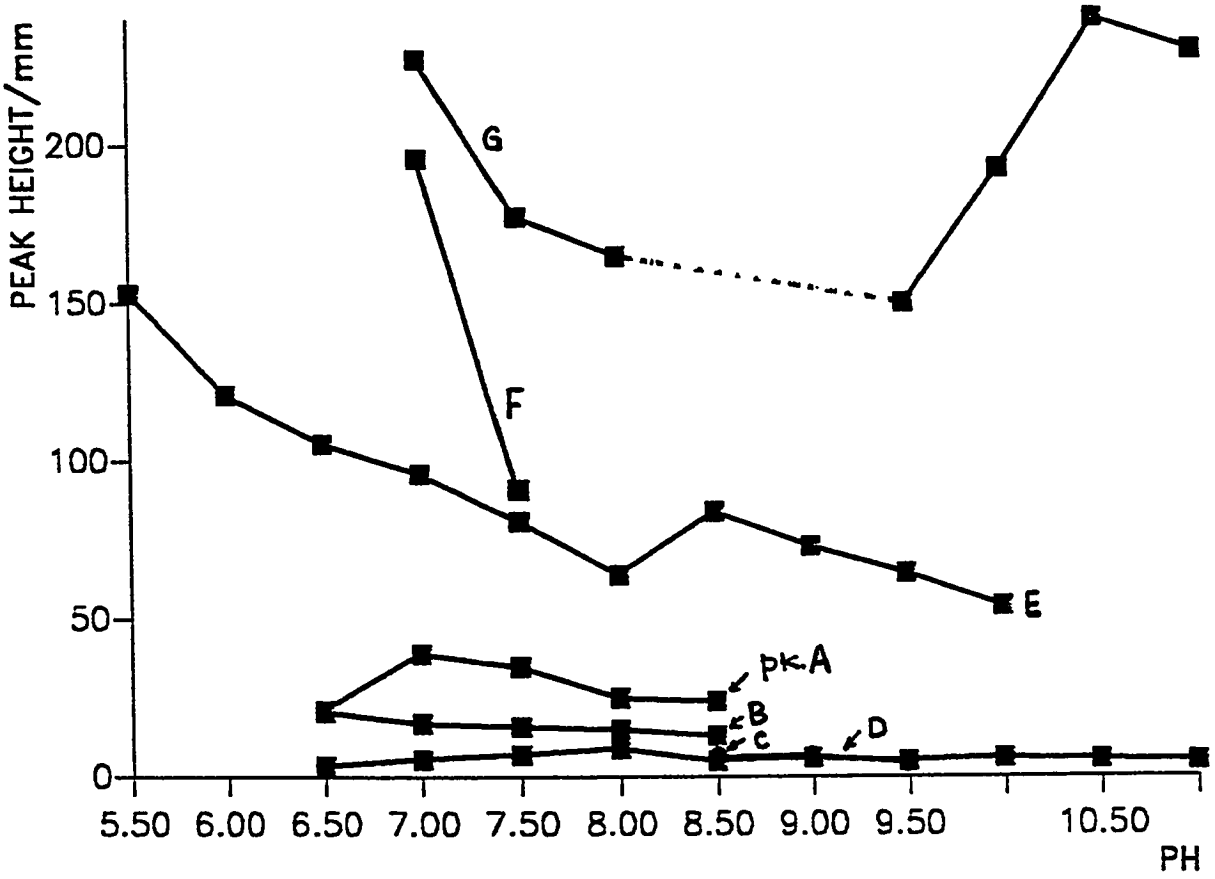


Fig. 4.7 CV peak heights vs. pH for 1×10^{-4}M norfloxacin in various buffering systems made of HOAc / NaOAc and NH_3 / NH_4Cl covering pH of 3.5 - 11.0. Peak C showed only once at pH 8.5. Scan rate used was 100 mV/sec and current sensitivity was 2 μA. The dashed line indicates the disappearance of peak G.

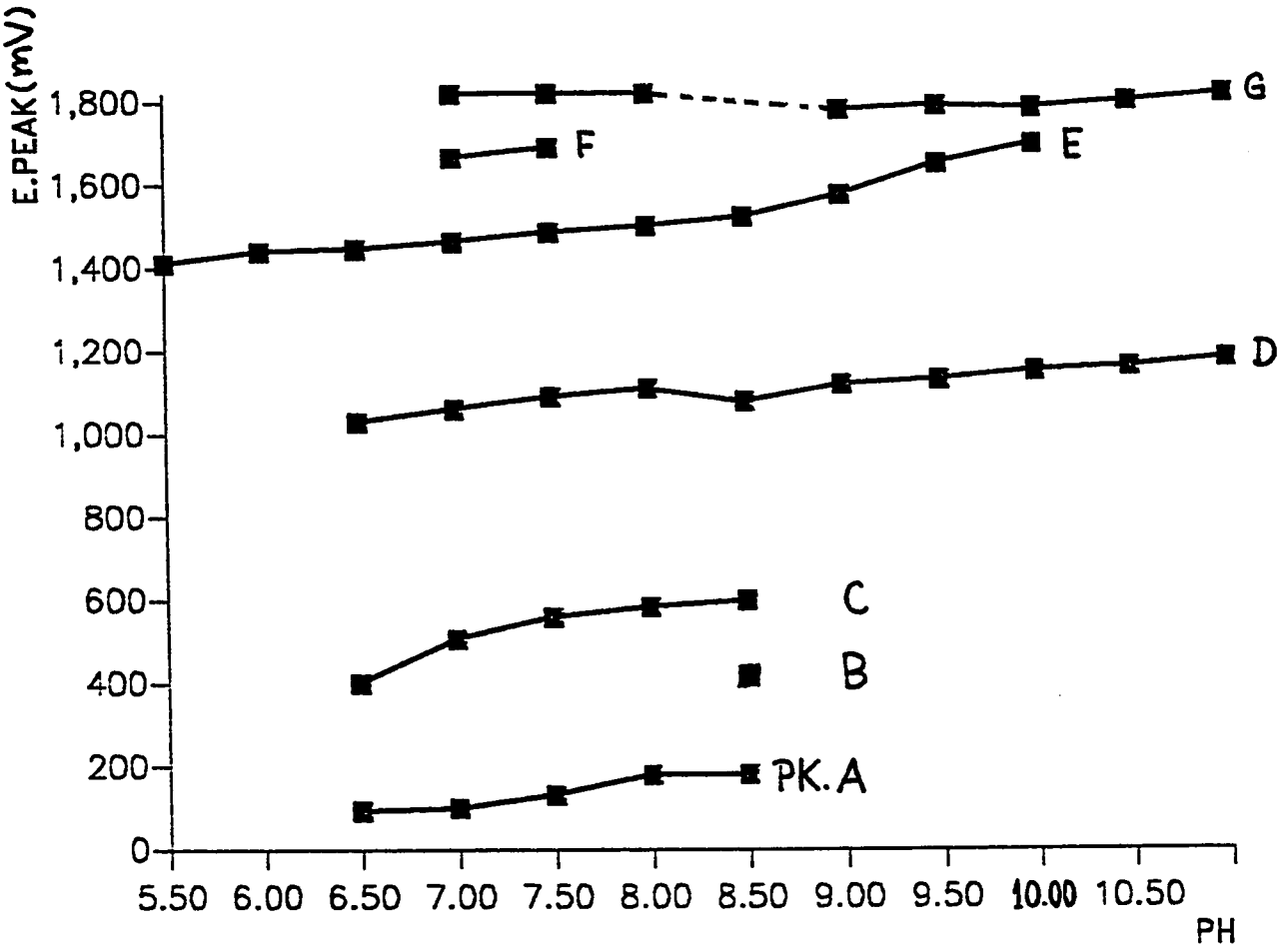
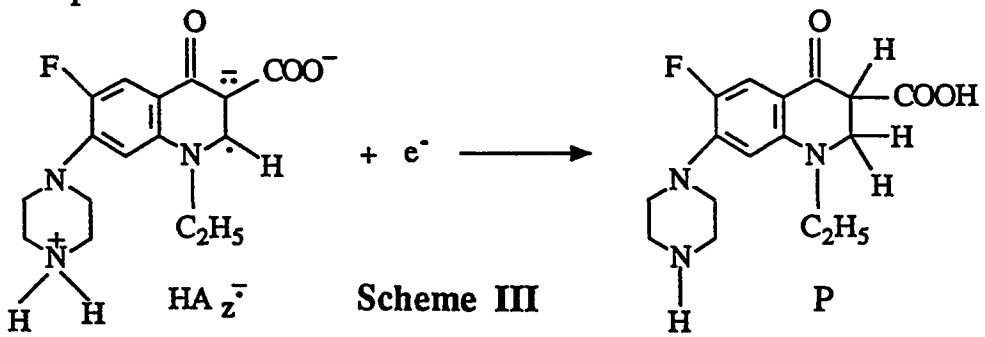


Fig. 4.8 CV peak potentials vs. pH for 1×10^{-4}M norfloxacin in various buffering systems made of HOAc / NaOAc and NH_3 / NH_4Cl covering pH of 3.5 - 11.0. Peak C showed only at pH 8.5. Scan rate used was 100 mV/sec and current sensitivity was 2 μA. The dashed line indicates the disappearance of peak G.

antibacterial drugs of the same type, namely, nalidixic acid (26), ciprofloxacin (27) and flumequine (29) showed a similar behavior using dc and dpp polarography. Also, this behavior is reported in chapter two using the dpp technique. The shift of Ep with pH towards more negative potential may indicate that the electron uptake is preceded by a proton transfer as proposed earlier for nalidixic acid (6).

Wave A showed a cathodic shift with pH without a break in its E_p-pH curve (Fig 4.8). Its E_p/pH value was found to be 43.0 mV below pH 8.5. This behaviour has been seen with the main wave at pH below 8.5. Due to the similarity between the two waves, a plausible mechanism is suggested, such that, the reduction of norfloxacin may take place in two one electron steps and the final product of the electrode reaction is the dihydrocompound where two hydrogens will be introduced to the double bond in the azinone ring as suggested for nalidixic acid (26). Thus, the proposed mechanism in chapter two may be incomplete. The radical ($HA_z^{\cdot}$) obtained may undergo another one electron electroreduction step to give the product as shown in scheme III.



Scheme III

Wave C also showed a cathodic shift with pH without a break in its E_p - pH curve (Fig 4.8). It is found that its E_p / pH is 63.8 mV below pH 8.5. Wave D is very small and was not observed with the dpp technique, hence it is neglected. However, wave B was observed only at pH 8.5.

The three waves A, B and C are similar in shape (adsorptive waves as mentined in chapter 2) and height and all showed different degree of reversibility (showed different ΔEp. Moreover, they are close to each other.

4.3.2 Effect of pH on the Cyclic Voltammetric Behavior of Nalidixic Acid

Cyclic voltammograms obtained for 1.0 x 10^{-4} M solutions of nalidixic acid of different pH values showed behavior similar to those obtained previously using dc polarography (26) and dp polarography performed in this study (chapter two). Nalidixic acid showed two reduction waves at potentials in the ranges of -0.80V to -0.94V and -1.10V to -1.19V in 0.01M, 0.1M, 1M and 2M HCl base electrolyte. Both waves have diminished in height and became flat (shoulder shape) at a position very close to the base electrolyte discharge curve in 2M HCl. However, in 0.01M HCl and with 200 mV/sec scan rate, the second wave splitted into two smaller waves which appeared at potentials of -1.0V and -1.16V. When the pH has been raised to higher values, the above mentioned reduction waves have been replaced by six different waves scattered in the range of -0.14V to -1.90V appearing at different pH values. At pH 4.0, three waves 1, 2 and 4 appeared at -0.17V, -1.05V and in the range of -1.24V to -1.28V, respectively (Fig. 4.9). Wave 1 was of negligible height at this pH; it has been observed better with scan rates ≥ 200 mV/sec. Waves 2 and 4 are the same waves seen in HCl with a small cathodic shift. It was observed that wave 2 was the main wave at certain pH, then wave 4 became the main wave in other pH. In the range of pH 4.5 to 6.0, a

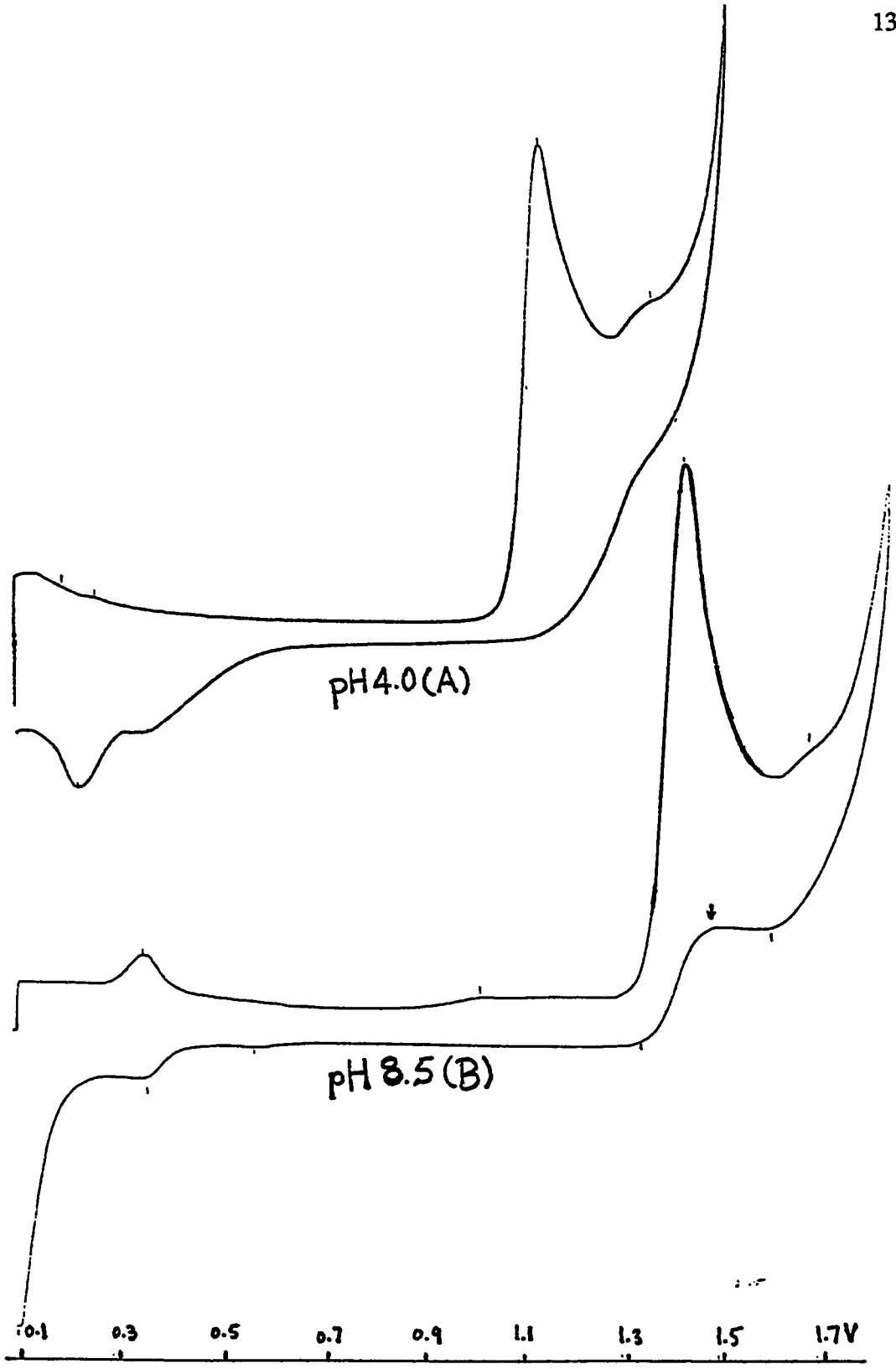


Fig. 4.9 Cyclic voltammograms for 1×10^{-4}M nalidixic acid in (A) HOAc / NaOAc pH, 4.0 (B) NH_3 / NH_4Cl pH 8.5. Scan rate used was 100 mV/sec. Current sensitivity was 2 μA.

post-shoulder was seen for wave 2 in the range -1.13V to -1.18V with a scan rate of 50 mV/sec. At pH 6.5 wave 2 has diminished in height with a scan rate of 50 mV/sec and its post-shoulder became a peak (wave 3) at -1.25V (Fig. 4.3, CV.B). In the range of pH 7.0 to 8.0, the maximum number of waves (five waves) been recorded in (Figure 4.3, Voltammogram C). A new peak (wave 6) has appeared at pH 7.0, in the potential range of -1.80V to -1.84V. It has diminished in height and became a shoulder in the range of pH 7.5 to 8.0. Also, at pH 7.0 wave 2, the main wave diminished in height and became a shoulder like for wave 3 which became by itself a shoulder like for wave 4, the main wave at this pH as observed from (Fig. 4.3, CV.C) and when the pH has reached 8.0, wave 2 has almost disappeared. It became a flat shoulder hardly seen for wave 3 which took a better shape of a peak and became the second main wave after wave 4 at this pH (Fig. 4.3, CV.C).

At pH 8.5, a new small negligible peak (wave n) appeared in the range -0.98 to -1.18V. Wave 1 became smaller and sharper and disappeared at pH 9.0. In the range of pH 8.5 to 11.0, nalidixic acid showed a behavior similar to that observed in HCl of concentration $< 1M$ where wave 2 appeared again with a huge size as the main wave and wave 5 decreased in size to be the second main wave as shown in (Fig. 4.4, CV.2). In this range of pH, the height of wave 2 was observed to be inversely proportional to pH. At pH 10.5 the main wave (wave 2) has decreased in height and became a shoulder for wave 4 which increased in height and became the main wave. Finally at pH 11.0, the shoulder fused completely with wave 4 to give one huge peak (wave 4) at -1.66V. At this pH only

two waves remained, wave 4 at -1.18V and wave n. When 0.1M sodium hydroxide was used as base electrolyte, only wave 4 remained at -1.77 V. It decreased in height and became more closer to the base electrolyte discharge curve and when 1M NaOH was used the wave increased in height but it became a shoulder very close to the base electrolyte discharge curve concentrations signifying its disappearance in sodium hydroxide of a concentration above 1M.

In the anodic scan nalidixic acid exhibited three catalytic waves at different pH values and potential positions. The size and clarity of these waves were noted to be dependant on pH. In various concentrations of HCl (1M, 0.1M and 0.01M), one ill-defined wave was noticed in the range of -1.04V to -1.13V. When the pH was raised to higher values, it was found that the size and clarity of the wave are proportional to pH. When the pH reached 6.5, the above mentioned wave became clear at -1.38V. From pH 3.5 to 6.5, this wave exhibited a shift of 160mV to more negative potential with pH. In the range of pH 7.0 to 8.0, the previously mentioned wave has been replaced by three clear catalytic waves (Fig. 4.3, voltammograms A & C, waves 4, 5, and 6) in the ranges of -1.40V to -1.43V, -1.56V to -1.60V and -1.86V to -1.92V. And when the pH reached 8.5, the three catalytic waves have been replaced by one well defined catalytic wave at -1.48V (Fig. 4.9, voltammogram B). In the pH range of 8.5 to 11.0, the size of this wave was found to be inversely proportional to pH, and it exhibited a shift of 120 mV to more negative potential. At pH 11.0, it became a shoulder appeared at -1.60V. When 0.1M sodium hydroxide was used as base electrolyte, the catalytic wave also exhibited a cathodic shift and appeared very close to the base electrolyte discharge curve at -1.85V.

And when 1M NaOH was used, the catalytic wave almost disappeared.

Fig. 4.10 shows the change in the peak heights of the five best defined waves 1, 2, 3, 4 and 5 with pH. The size of wave 2 decreases with increasing pH in the shape of a dissociation curve with an inflection point at pH 6.2. This pH is considered to be the polarographic pKa for wave 2 ($pK'i_2$) for nalidixic acid which is in good agreement with the value 6.02 - 6.11 mentioned in the literature (26). Attribution of the decrease of wave 3 in the range of pH from HCl 1M to pH 8.0 to the dissociation of the carboxyl group would involve $pK'i_2 \approx pK_{COOH}$ (26). Such agreement implies an acid-base equilibrium established slowly when compared with the time window (3 sec) of a polarographic measurement (26).

Wave 5 showed a break (Fig. 4.10) of minimum peak height at pH 8. This break may be attributed to a change in the mechanism of the electrochemical-reduction process at this pH which may be due to the change in the base electrolyte from NaOAc (pH 8.0) to NH_3/NH_4 Cl (pH 8.5).

Looking at Fig. 4.10, we observe that if the i_p - pH plot for wave 3 is extrapolated beyond pH 8 as has been done in the case of the previous (26) dc study, we get another dissociation curve for wave 3 with an inflection point at pH 8.5 which is also considered as the polarographic pKa for nalidixic acid for wave 3 ($pK'i_3$). This value is also in good agreement with the one obtained previously for nalidixic acid (26). Attribution of the decrease of wave 3 with pH in the range from 1M HCl to pH 8.0 to the dissociation of the carboxyl group resulted in $pK'i_3 > pK$ COOH, because

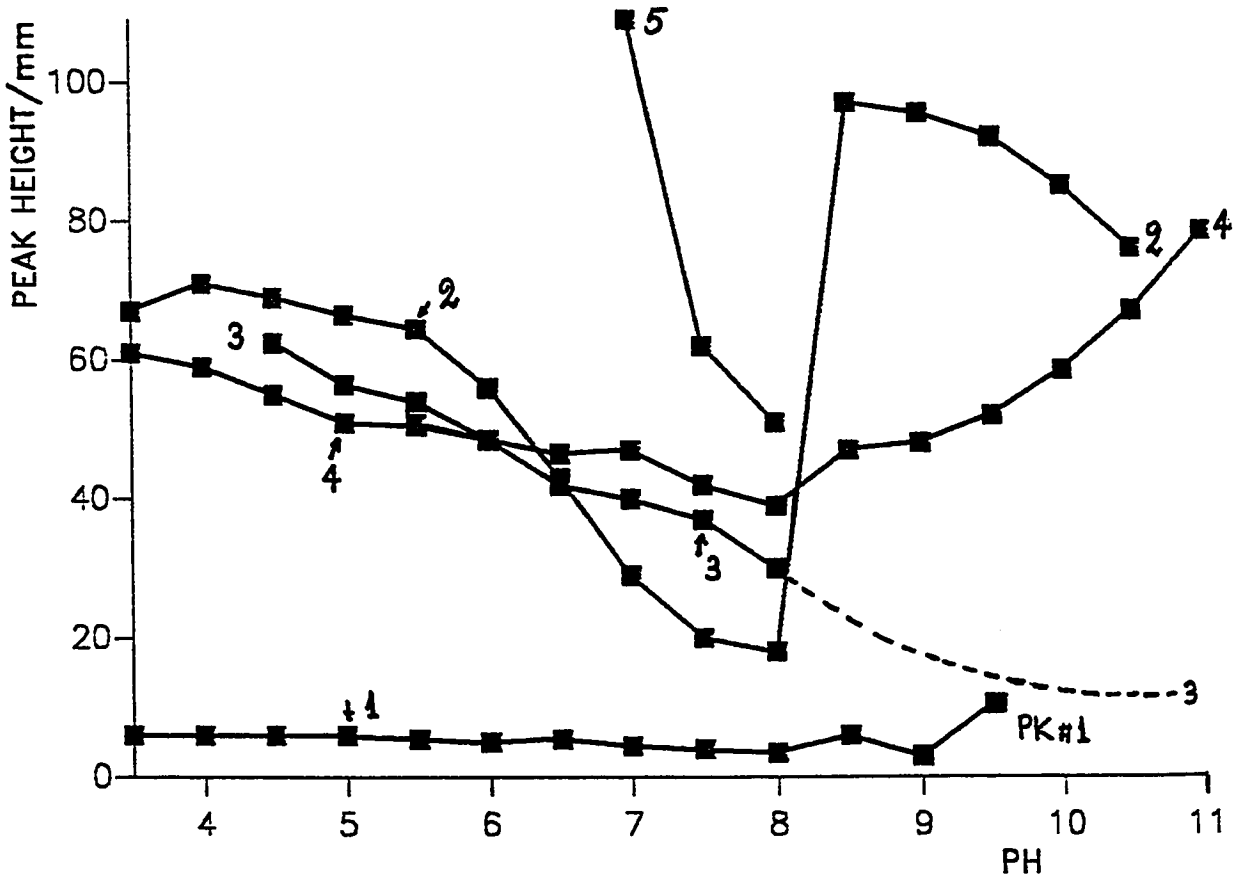


Fig. 4.10 CV peak heights vs. pH for 1×10^{-4}M nalidixic acid in various buffering systems made of HOAc / NaOAc and NH_3 / NH_4Cl covering pH of 3.5 - 11.0. Scan rate used was 100 mV/sec and current sensitivity was 2 μA.

of recombination of anions with proton donors in the vicinity of the electrode (26). The inflection points of polarographic dissociation curves (pK') for reducible acids are shifted to higher pH values than the thermodynamic pK values (3, 26). Thus, the decrease in the height of waves 2 and 3 with increasing pH at about 8.0 can be attributed to the dissociation of the carboxyl group.

The reaction scheme can be described by the acid-base equilibrium between species A and B given by rection (1) followed by reactions (2-9) shown in scheme IV. Waves 2 to 6 are assigned to their proper reactions as observed in scheme IV. The decrease in wave 2 with pH at pH > 5 may be ascribed to the decrease in the rate of protonation of species B in reaction (1). It was concluded that the protonation of species B resulting in the formation of A occurs on the ethylenic bond (26), and this proton transfer reaction may be responsible for the cathodic shift of 59.59 mV/pH of wave 2 (Fig. 4.11) over the range of pH 3.5 to 8.0. This value is in good agreement with the value reported (61 mV) previously (6).

The shift in the potential of wave 4 by 66.75 mV/pH in the pH range below pH 8.0 may indicate the presence of a proton transfer (reaction 6) between the first and the second electron uptake. Wave 3 which replaces wave 2 at pH > 6 may correspond to the reduction of species B (26). A decrease in the rate of protonation of the anion species C to give the neutral species B may result in a decrease in the height of wave 3 at pH > 7 (Fig. 4.10). Shifts of peak potentials of wave 3 by 73 mV/pH which is comparable to the one obtained previously (26) 63 mV/pH, indicates

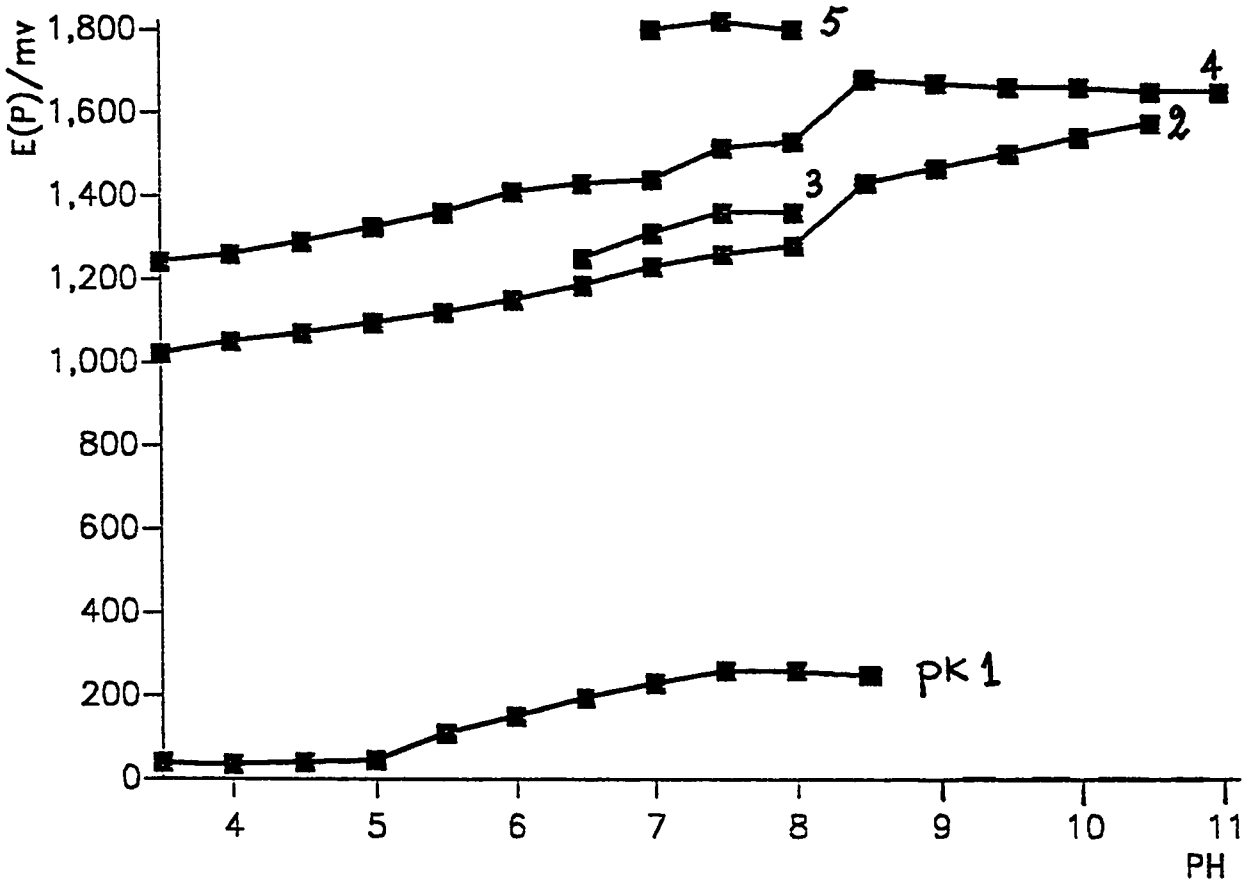
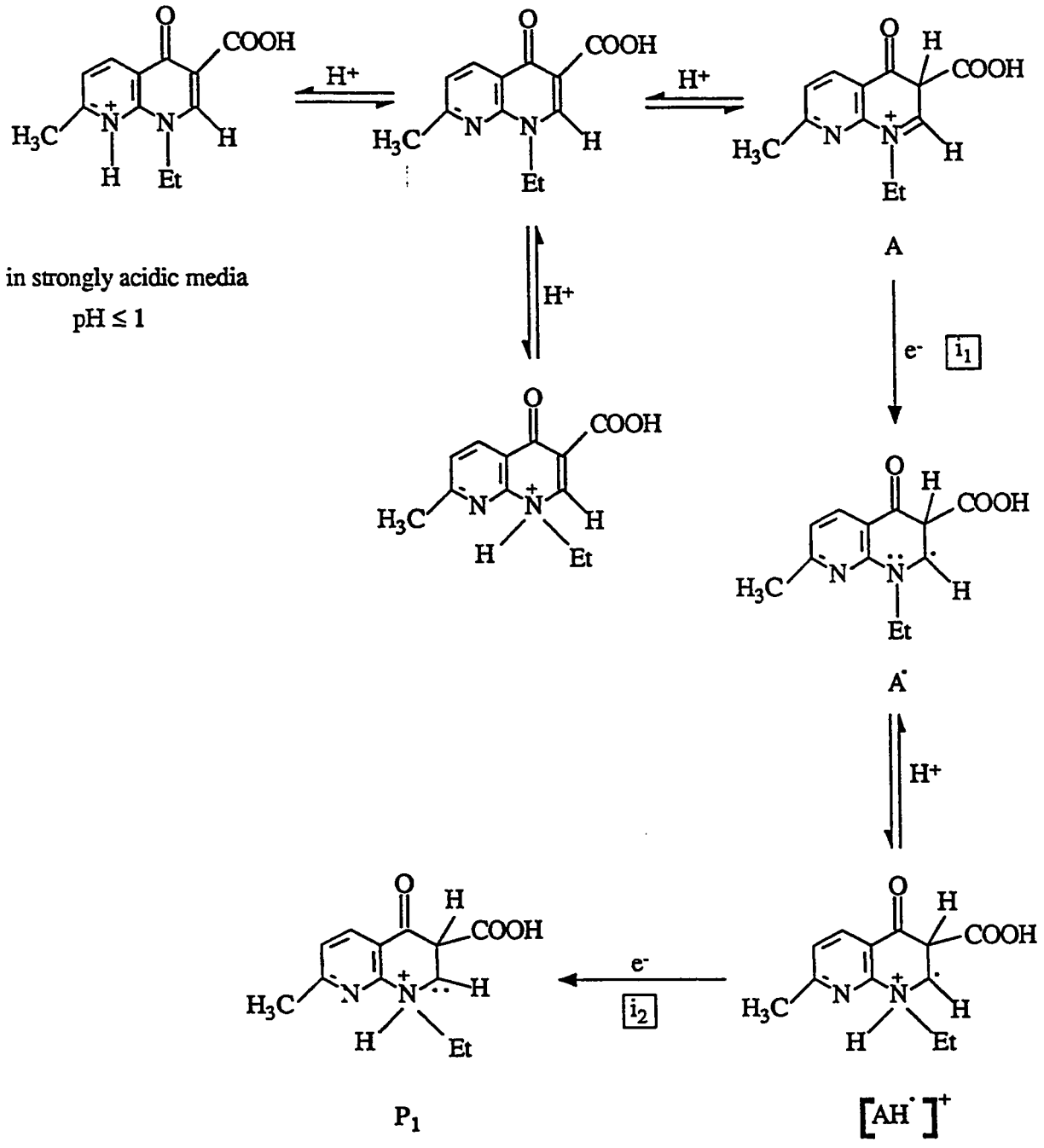
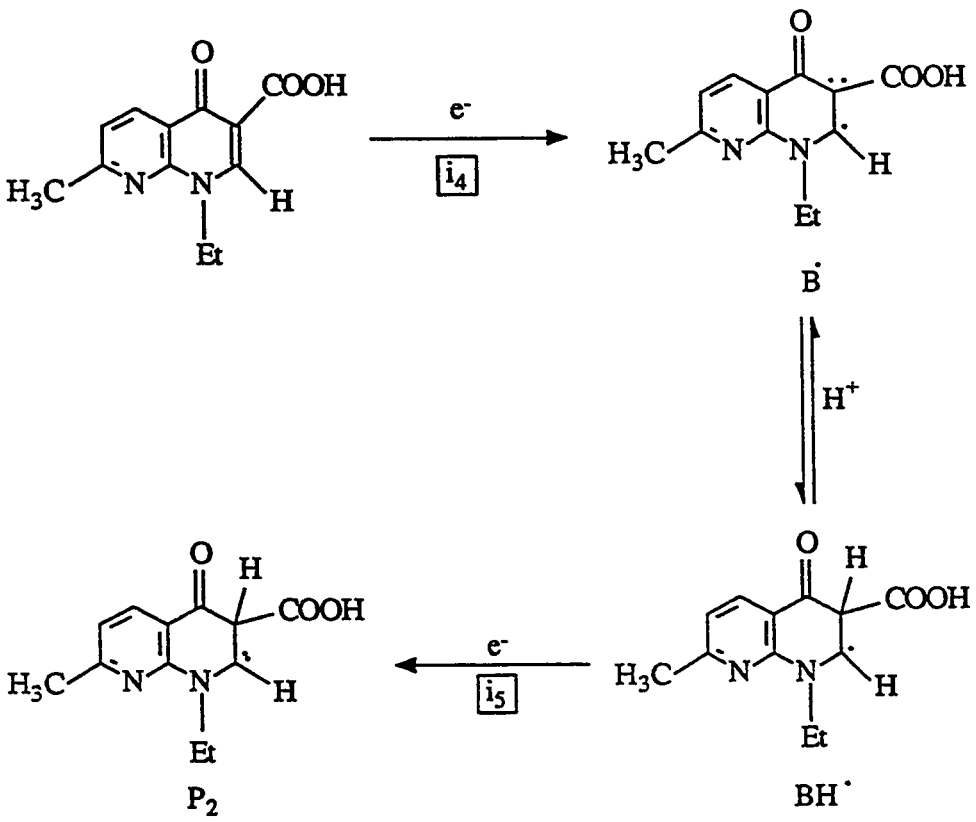


Fig. 4.11 CV peak potentials vs. pH for 1×10^{-4}M nalidixic acid in various buffering systems made of HOAc / NaOAc and NH_3 / NH_4Cl covering pH of 3.5 - 11.0. Peak C showed only at pH 8.5. Scan rate used was 100 mV/sec and current sensitivity was 2 μA.

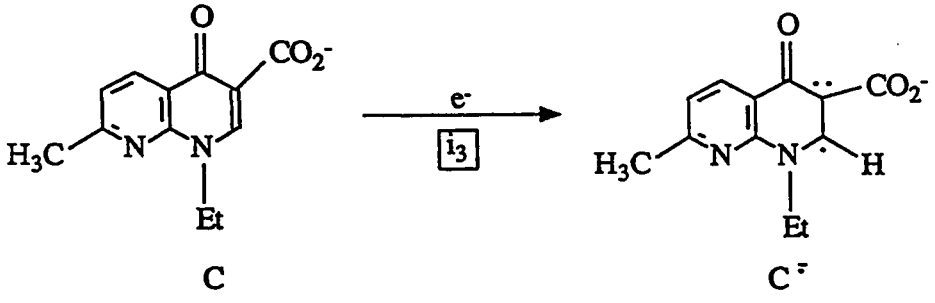
Scheme IV : Electrochemical reduction of nalidixic acid at the HMDE



pH ≈ 6 - 8



pH ≈ 10 - 13



protonation of the anion C (which predominates in this pH range in the bulk of the solution) in reaction (4) preceding the first electron uptake in reaction (8). Moreover, the shift in the potentials of wave 5 by 50.4 mV/pH is also in good agreement with the reported (26) value (54 mV/pH) indicates another proton transfer (reaction 9) occurring between the first and the second electron transfer (reactions 8 and 10). Wave 6 corresponds to a one electron reduction (reaction 11) of the least protonated species C. Since the species C is predominant at pH > 8.5 the peak potential of wave 6 is pH independant, because reaction (11) is not preceded by a proton transfer.

4.4 Relationship Between Current Function and Scan Rate

According to the Randles - Sevick equation (1) for reversible processes

$$i_p = 269\, n^{3/2}\, A\, D^{1/2}\, V^{1/2}\, C$$

and for irreversible processes

$$i_p = 269\, n(\alpha n_\alpha)^{3/2}\, A\, D^{1/2}\, V^{1/2}\, C$$

where i_p is the peak current in microamperes, n is the number of electrons involved in the electrode process, α is the charge transfer coefficient, A is the area of the mercury drop in cm^2, D is the diffusion coefficient in cm^2/sec, V is the scan rate in volts/second and C is the bulk concentration in moles/liter.

The relationship between the limiting current and the scan rate is simply indicated by the plot of $i_p / V^{1/2}$ versus scan rate (equation above), which decides the various electrode processes (44, 45). If an electrode process is diffusion controlled, the current function $i_p / V^{1/2}$ will be independant of scan rate. In the case of adsorption controlled process, $i_p / V^{1/2}$ increases with increasing scan rate.

For an adsorption controlled process, peak current is directly proportional to the scan rate (V), and a cathodic shift may be observed with scan rate (4).

LSV and CV may be used to study the adsorption of the electrochemical reactant or product at the electrode surface. Adsorption may cause enhancement of the peak currents or splitting of waves and appearance of separate adsorption peaks prior to or after the normal peak (4). It has been reported that three tests can be performed to confirm the presence or absence of adsorption in an electrochemical process, namely (4):

a. adsorption waves are frequently symmetrical unlike the normal waves.

b. $i_p/(C_o^* V^{1/2})$ generally increases reapidly with increasing scan rates. However, $i_p/(C_o^* V)$ may remain nearly constant.

c. i_p/C increases with the decrease in concentration which may be levelled off to a constant value for low concentrations.

The peak currents for norfloxacin and nalidixic acid were plotted versus scan rate (V) and versus the square root of the scan rate ($V^{1/2}$). The current function $i_p / V^{1/2}$ is also plotted versus scan rate. Most of the peaks showed by norfloxacin exhibited adsorptive characteristics. Peaks 1 and 2 were found to be adsorption controlled with scan rates ≤ 50 mV/sec. At higher scan rates, they were found to be diffusion controlled. However, peaks 3, 4 and 5 exhibited only adsorption characteristics. Peak 6 which is the main peak was also found to be adsorption controlled with scan rates ≤ 200 mV/sec. For instance, it was found experimentally, that norfloxacin adsorbs best in LiCl base electrolyte with scan rates < 20. The adsorption was optimum with scan rate of 2 mV/sec, where it gave the biggest peak current; however, when the scan rate was increased to higher values, the peak current decreased dramatically. Peak 7, at the most negative potential, and the peak observed in 2M HCl were found to be diffusion controlled.

However, most of the peaks showed by nalidixic acid were found to be diffusion controlled. The first one, at the most positive potential exhibited adsorptive behavior. It was not reported previously in the dc polarographic study (26) due to its insignificance there. However, the other peaks, 2, 3, 4, 5 and 6 were found to be diffusion controlled. Peak 2 showed adsorption characteristics with scan rates > 20 mV/sec in slightly acidic solutions as in the case of KCl (pH 5.8) and in LiCl (pH 5.7) base electrolytes. This peak was also previously reported (26) in the dc polarography to be adsorptive in slightly acidic pH. Also, the first peak

(main peak) exhibited by nalidixic acid in 1M HCl was found to be controlled by adsorption, however, the second peak is controlled by diffusion. The results obtained for nalidixic acid do agree well with the previous dc polarography study reported in the literature (26). The results obtained for the different electrode processes assigned to the different peaks exhibited by norfloxacin and nalidixic acid in various base electrolytes are summarized in Tables 4.4 and 4.5.

Table 4.4: Determination of the nature of the electrode process for 1 x 10^{-4}M norfloxacin in different base electrolytes using the relationship between limiting current and scan rate derived from the linear scan voltammetry.

Base Elect. and Peak #	$-E_p$ (V)	E_p Shift with V	i_p Vs. V r	Scan rate range (mV/sec)	i_p vs. $V^{1/2}$ r	Scan rate range (mV/sec)	$i_p/V^{1/2}$ vs V notes	Electrode Process
PK = 1								
NaOAc	0.15	Cathodic	0.9970	5 - 50	0.9991	50 - 200	Proportional upto 50 mV/sec then levels off, then decreases	Ads. Contr. V ≤ 50 diff. Contr. V > 50
KCl	0.10	Cathodic	0.9940	5 - 50	curved	50 - 200	Proportional upto 50mV/sec then inversely proportional	Ads. Contr. process V ≤ 50
LiCl (Forward)	0.14	Cathodic	0.9996	5 - 20	0.9997	20 - 100	Proportional upto 20mV/sec then inversely proportional	Ads. Contr. V ≤ 20 diff. Contr. V > 20
LiCl (Backward)	0.09	Anodic	0.9993	5 - 200	upward incline	5 - 200	Proportional	Adsorpt controlled process
PK = 2								
K3 Citrate	0.23	Cathodic	0.9972	5 - 50	0.9997	50 - 500	Proportional upto 100mV/sec then levels off, then decreases at 500 mV/sec	Ads. Contr V ≤ 50 diff. Contr. V > 50
PK = 3								
LiCl (Forward)	0.35	Cathodic	0.9944	5 - 50	upward incline	5 - 500	Proportional upto 500mV/sec	Ads. Cont. process
LiCl (Backward)	0.28	Anodic	0.9990	5 - 200	0.9995	100 - 500	Proportional upto 200mV/sec then became inversely proportional	Ads. Contr V < 200 diff. Contr. V > 200
KCl	0.36	Cathodic	0.9994	5 - 50	upward incline	5 - 500	proportional	Ads. Contr. process

PK = Peak

.....Contd/ 2,

Base Elect. and Peak #	$-E_p$ (V)	E_p Shift with V	i_p Vs. V r	Scan rate range (mV/sec)	i_p vs. $V^{1/2}$ r	Scan rate range (mV/sec)	$i_p/V^{1/2}$ vs V notes	Electrode Process
PK = 4								
NaOAc	0.57	Cathodic	0.9990	5 - 200	upward incline	5 - 500	Proportional	Ads. Contr. process
K_3 Citrate	0.61	Cathodic	0.9990	5 - 200	upward incline	5 - 500	Proportional	Ads. Contr. process
KCl	0.55	Cathodic	0.9998	5 - 500	upward incline	5 - 500	Proportional	Ads. Contr. process
LiCl	0.44	Cathodic	0.9999	5 - 200	upward incline	5 - 200	Proportional	Ads. Contr. process
PK = 5								
LiCl (Forward)	1.22	Cathodic	0.9998	5 - 200	upward incline	5 - 1000	linearly proportional r = 0.9998	Ads. Cont. process
(Backward)	1.00	Anodic	0.9999	200-1000	0.9999	50 - 500	linearly proportional V > 200mV/sec	Diff. Contr. V ≤ 200 Ads. Contr. V > 200
PK = 6 (Main) NaOAc	1.49	Cathodic	0.9975	5 - 200	0.9999	200-1000	Proportional from 20 - 200 then levels off at V>200mV/sec	Ads. Contr V ≤ 200 diff. Contr. V > 200
K_3 Citrate	1.54	Cathodic	0.9975	50 - 200	0.9999	200-1000	Proportional from 50-200 then levels off at V>200mV/sec	Ads. Contr. V, 50-200 diff. Contr. V> 200
$K_2B_4O_7$	1.60	Cathodic	0.9920	50 -200	0.9980	5 - 50	Inversely Proportional from 5 - 50, then prop. from 50 - 200	diff. Contr process V ≤ 50 Ads. Contr. V > 50

.....Contd/ 3,

Base Elect. and Peak #	$-E_p$ (V)	E_p Shift with V	i_p Vs. V r	Scan rate range (mV/sec)	i_p vs. $V^{1/2}$ r	Scan rate range (mV/sec)	$i_p/V^{1/2}$ vs V notes	Electrode Process
KCl	1.51	Cathodic	0.9990	5 - 200	0.9998	100 - 500	Proportional from 20-200 mV/sec then levels off	Ads. Contr. V ≤ 200 diff. Contr. V > 200
LiCl	1.56	Cathodic	0.9950	5 - 20	0.9992	50 - 500	proportional from 5-20 mV/s then inversely proportional then levels off.	Ads. Contr. V ≤ 20 diff. Contr. V > 50
HCl (2M)	1.02	Cathodic	curved	5 - 50	0.9992	5 - 50	inversely proportional	diff. Contr. process
PK = 7 (Last PK)								
K_3 Citrate	1.80	Anodic	Curved	5 - 200	0.9994	5 - 200	nversely proportional	diff. Cont. process
KCl	1.80	Const.	curved	5 - 200	0.9995	5 - 200	inversely proportional	diff. Cont. process

Table 4.5: Determination of the nature of the electrode process for 1 x 10^{-4} M nalidixic acid in different base electrolytes using the relationship between limiting current and scan rate derived from the linear scan voltammetry.

Base Elect. and Peak #	$-E_p$ (V)	E_p Shift with V	i_p Vs. V r	Scan rate range (mV/sec)	i_p vs. $V^{1/2}$ r	Scan rate range (mV/sec)	$i_p/V^{1/2}$ vs V notes	Electrode Process
PK = 1								
NaOAc	0.16	Cathodic	0.9996	5 - 500	upward incline	50 - 1000	Proportional	Ads. Contr. process
K_3 Citrate	0.18	Const.	0.9999	10-1000	upward incline	5 -1000	Proportional	Ads. Contr. process
KCl	0.19	Cathodic	0.9997	5 - 500	upward incline	5 - 500	Proportional	Ads. Contr. process
PK = 2								
KCl	1.12	Cathodic	0.9994	5 - 200	0.9990	5 - 20	5-20 inversely proportional and V>20 prop.	diff. Contr V < 20 Ads. Contr. V > 20
LiCl	1.11	Cathodic	0.9999	50-200	0.9999	5 - 20	5-20 inv. proportional and V > 20 prop.	diff. Contr. V < 20 Ads. Contr. V > 20
PK = 3								
NaOAc	1.35	Const.	0.9999	200-1000	0.9995	5 -200	inv. propor. V = 5-200 prop. where V > 200mV/sec	diff. Const. process V = 5-200 then Ads Cont at V > 200
K_3Citrate	1.36	Const.	0.9999	100-1000	0.9994	5 - 100	inv. prop. V = 5-100, then prop. V> 100	diff. Contr. V = 5-100 then Ads. Contr. V>100

.....Contd/ 2,

Base Elect. and Peak #	$-E_p$ (V)	E_p Shift with V	i_p Vs. V r	Scan rate range (mV/sec)	i_p vs. $V^{1/2}$ r	Scan rate range (mV/sec)	$i_p/V^{1/2}$ vs V notes	Electrode Process
PK = 4 NaOAc	1.49	Const.	curved	5 - 1000	0.9999	5 - 1000	inv. prop. upto 100mV/sec then Const.	diff. Contr. process
K_3 Citrate	1.56	Cathodic	curved	5 - 500	0.9999	5 - 500	inv. prop.	diff. Contr. process
K2B4O7	1.61	Cathodic	curved	5 - 500	0.9991	5 -500	inv. prop.	diff. Contr. process
KCl	1.50	Anodic	curved upto 200 >200 mv /sec lin.	5 - 1000	0.9980	5 - 100	inv. prop. from 5 - 100 then prop. where V > 100 mv/sec	diff. Contr. V ≤ 100 Ads. Contr. V > 100
LiCl	1.50	Anodic	0.9920	100-1000	0.9970	5 - 100	inv. prop. from 5 - 100 then linearly prop. V > 100	diff. Contr. from 5 -100, Ads. Contr. V > 100
PK = 5 NaOAc	1.78	Cathodic	curved	20 - 500	0.9990	20 - 500	inv. prop. from 20 to 200 then const. upto 500 mV/sec.	diff. Const. process
K3 Citrate	1.76	Cathodic	0.9993	50 - 500	0.9992	5 - 50	inv. prop. from 5 -50 then prop. linearly from 50 - 500	diff. Contr. V ≤ 50 Ads. Contr. V > 50
K2B4O7	1.75	cathodic	curved	5 - 1000	0.9996	10 -1000	inv. prop from 10-50 then almost const. where V > 50 mv/sec	diff. Const. process

.....Contd/ 3,

Base Elect. and Peak #	$-E_p$ (V)	E_p Shift with V	i_p Vs. V r	Scan rate range (mV/sec)	i_p vs. $V^{1/2}$ r	Scan rate range (mV/sec)	$i_p/V^{1/2}$ vs V notes	Electrode Process
KCl	1.76	cathodic	curved	5 - 1000	0.9995	10 - 1000	inv. prop. from 10-50 then const. where V>50 mV/sec	diff. Contr. process
LiCl	1.72	Cathodic	curved	5 - 1000	0.9995	50 - 1000	inv. prop. from 5-100 then almost stayed const.	diff. Contr. process
HCl (1M) Main Peak	0.81	Cathodic	0.9997	5 - 200	upward incline	5 - 200	proportional	diff. Contr. process
Last Peak	1.06	Anodic + Cathodic	curved	5 - 500	0.9997	5 - 1000	inversely proportional	diff. Contr. process

Note: Peak # 6, at the most negative potential (from -1.86V - 1.90V) is very flat and seen only with LiCl and KCl base electrolytes. From its shape, it is considered to be a diffusion peak as reported in the literature (26)

4.5 Determination of Reversibility/Irreversibility for the currents Exhibited by Nalidixic Acid and Norfloxacin

The degree of reversibility was tested by recording cyclic voltammograms for both nalidixic acid and norfloxacin. ΔEp and the ratio i_{pa}/i_{pc} were determined for each drug at different scan rates and in various base electrolytes. The data is shown in Table 6. The CV voltammograms obtained for $1x10^{-4}$ M of nalidixic acid in various base electrolytes showed six irreversible peaks at different potential positions as observed in Fig. 2. This irreversibility behavior agrees well with the mechanism previously proposed using dc polarography (26).

However, cyclic voltammogram for 1×10^{-4} M norfloxacin showed seven peaks in different base electrolytes. Only the first small and sharp peak at the most positive potential was found to be reversible with a number of electrons transferred n equal 2 (sections 4.2 & 4.3). Peaks B and C (Fig. 4.2) showed a sluggish reversibility. The main peak, and the other three peaks were found to be irreversible. The results for the peaks of norfloxacin that showed some degree of reversibility are summarized in Table 4.6.

Table 4.6: Determination of the peak potential separation, DEp and the ratio ipa/ipc for 1 x 10^{-4}M norfloxacin in various base electrolytes using CV. The current sensitivity was 2μA.

Base Elect. and Scan rate (mV/sec)	E_{Pc} (V)	E_{Pa} (V)	i_{Pa} (cm)	i_{Pc} (cm)	$\Delta E_{P(c-a)}$ (mV)	$\frac{i_{Pa}}{i_{Pc}}$	Notes
NaOAc							
50	0.140	0.077	0.80	1.30	63	0.62	slow rev.
100	0.153	0.090	2.10	2.00	63	1.05	reversible
200	0.150	0.090	3.30	3.30	60	1.00	reversible
50	0.563	0.453	0.75	0.75	110	1.00	slow rev.
100	0.570	0.450	1.85	1.25	120	1.08	slow rev.
200	0.577	0.457	2.20	2.20	120	1.00	slow rev.
K_3 Citrate							
50	0.220	0.180	1.00	1.40	40	0.71	slow rev.
100	0.230	0.183	2.00	2.20	47	0.91	reversible
200	0.240	0.180	3.60	3.20	60	1.12	reversible
50	0.600	0.510	0.20	0.60	90	0.33	irreversible
100	0.610	0.500	0.30	1.20	110	0.25	irreversible
200	0.617	0.490	0.45	2.10	127	0.21	irreversible
$(CH_3)_4$ NBr							
50	0.440	0.370	0.30	0.25	70	1.20	slow rev.
100	0.440	0.370	0.60	0.60	70	1.00	slow rev.
200	0.450	0.390	0.55	0.55	60	1.00	reversible
KCl							
50	0.100	0.060	1.70	2.80	40	0.61	slow rev.
100	0.103	0.057	3.40	3.40	46	1.00	reversible
200	0.108	0.063	3.50	3.50	45	1.00	reversible
50	0.353	0.273	1.20	0.70	80	1.71	slow rev.
100	0.360	0.270	2.10	1.30	90	1.62	slow rev.
200	0.370	0.267	3.45	2.00	103	1.72	slow rev.

Base Elect. and Scan rate (mV/sec)	E_{P_c} (V)	E_{P_a} (V)	i_{P_a} (cm)	i_{P_c} (cm)	$\Delta E_{P(c-a)}$ (mV)	$\frac{i_{P_a}}{i_{P_c}}$	Notes
KCl							
50	0.550	0.470	0.20	0.75	80	0.27	irreversible
100	0.550	0.480	0.70	0.70	70	0.29	irreversible
200	0.560	0.500	0.50	1.50	60	0.30	irreversible
LiCl							
50	0.140	0.083	1.50	1.75	57	0.86	reversible
100	0.143	0.083	2.90	2.60	60	1.12	reversible
200	0.150	0.097	3.00	3.10	53	0.97	reversible
50	0.350	0.280	2.0	1.00	70	2.00	slow rev.
100	0.353	0.277	3.10	1.60	76	1.94	slow rev.
200	0.360	0.277	5.00	2.50	83	2.00	slow rev.
50	---	---	---	---	---	---	---
100	1.220	1.000	0.30	0.65	220	0.46	irreversible
200	1.220	0.930	1.70	1.70	290	1.42	irreversible

Note: Peaks not mentioned here are completely irreversible.

4.6 Repetitive Cyclic Voltammograms of Norfloxacin and Nalidixic Acid

The repetitive cyclic voltammetry for norfloxacin and nalidixic acid was investigated at a hanging mercury drop electrode (HDME). Typical cyclic voltammetric voltammograms for norfloxacin and nalidixic acid are shown in Figs. 4.12 and 4.13. Norfloxacin showed a different behavior with the successive cycles compared to the first cycle. Using a scan rate of 100 mV/sec in the forward direction (cathodic scan), the first small and sharp peak at the most positive potential (wave A) decreased in height and exhibited a cathodic shift with more cycles. However, the second similar peak (wave B) observed at pH 8.5 only at a potential position of -0.39V kept constant in height and position. The third small and sharp peak which appeared with the first peak in slightly acidic to slightly basic solutions at -0.38V (wave C), also decreased in height and exhibited a small cathodic shift with the second cycle, then stayed constant in height and position. However, the fourth small and negligible peak (wave D) seen with LiCl base electrolyte at -1.23V showed an increase in peak current with more cycles at the same position. In the same base electrolyte (0.1M LiCl), the main peak started to disappear with more cycles by increasing in height and taking a shoulder shape. However, in NaOAc base electrolyte, the main peak started to disappear by taking a shoulder shape without increasing in height as observed from Fig. 4.12; and at the same time a new peak started to appear at -1.66V. With further cycles the main peak may disappear completely and the new peak may appear with a better shape. And finally, the last peak at the most negative potential (wave F) started to increase by taking a better shape of a peak with successive cycles (Fig. 11).

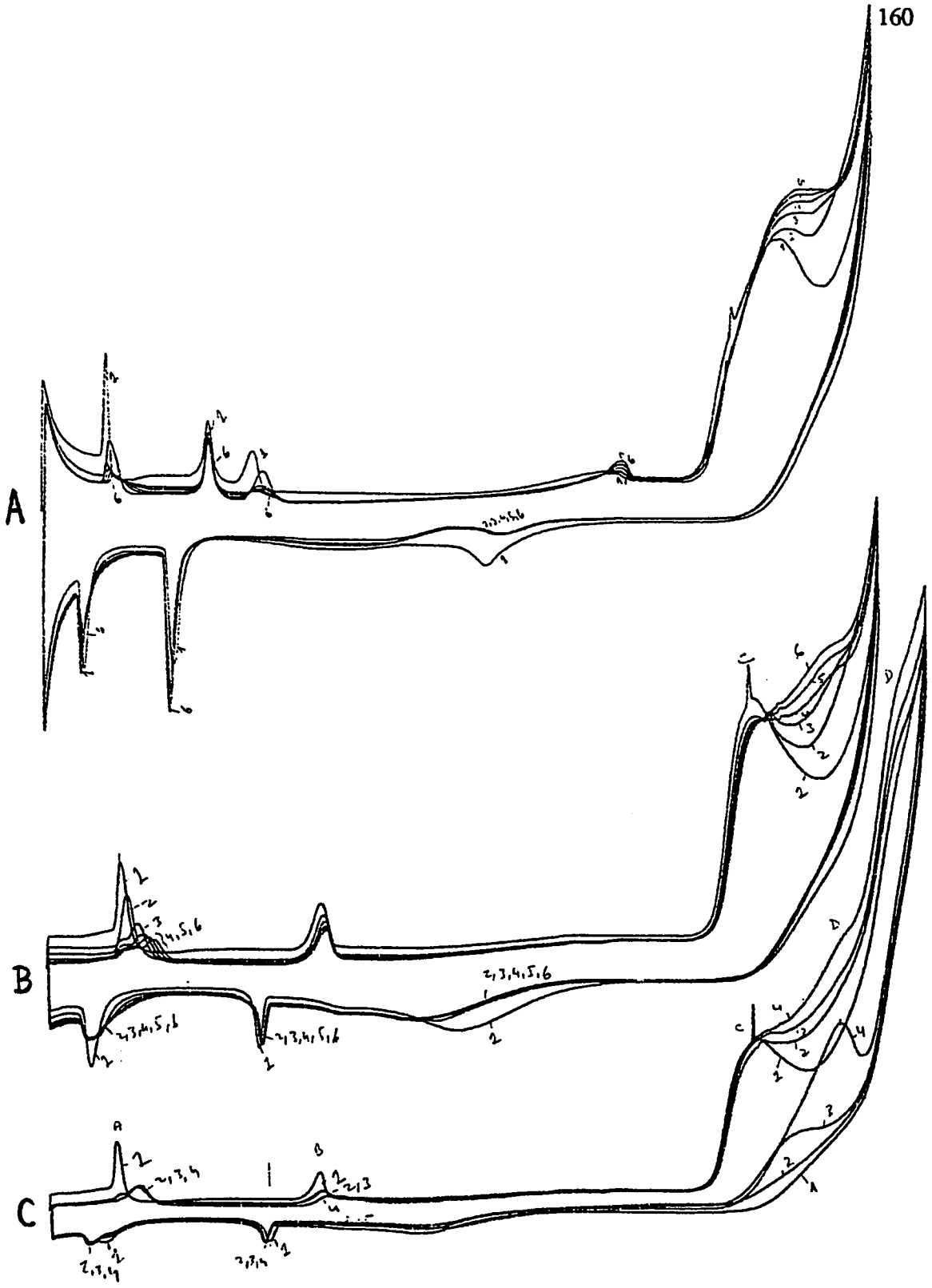


Fig. 4.12 Repetitive cyclic voltammograms for 1×10^{-4}M norfloxacin in 0.1M of base electrolytes. (A) LiCl, scan rate used was 100 mV/sec, n = 6 (B) NaOAc, v = 100 mV/sec, n = 6 and (C) NaOAc, v = 50 mV/sec, n = 4. Current sensitivity was 2 μA.

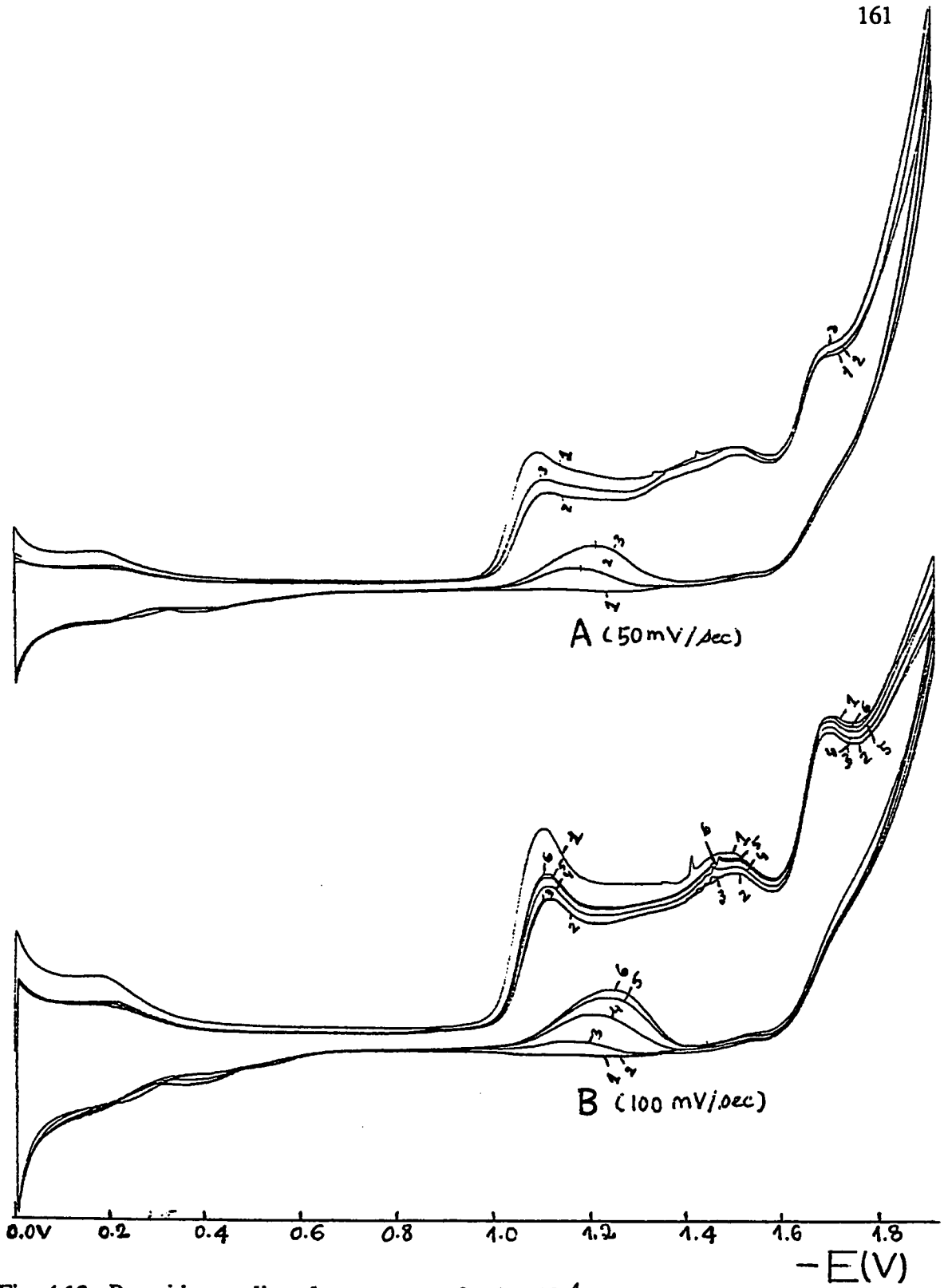


Fig. 4.13 Repetitive cyclic voltammograms for 1×10^{-4}M nalidixic acid in 0.1M LiCl base electrolyte. (A) Scan rate 50 mV/sec, n = 3 (B) scan rate 100 mV/sec, n = 6. Current sensitivity was 2 μA.

In the backward (anodic) scan, the first peak at the most positive potential (wave 1) has decreased in height and became wider with the second cycle, then kept constant in height and position. However, the next similar peak (wave 2) stayed almost constant in height and position with more cycles. The third small and wide anodic peak (wave 3) at 0.9V decreased in height with the second cycle then stayed constant in both height and position. When a scan rate of 50 mV/sec was used, a catalytic wave appeared at -1.56V in the third cycle with sodium acetate base electrolyte (Fig. 4.12). It increased in height and shifted to -1.67V with the fourth cycle. This catalytic wave indicates the presence of a catalytic process taking place slowly. The catalytic effect increased with more cycles which may be due to the formation of the catalytic species with time, as shown in Fig. 4.12.

All changes with more cycles compared to the first cycle (Fig. 4.12) may indicate that some other reactions may be coupled with the main electrode reaction. For instance, the decrease in the first peak in the anodic scan (wave 1), the disappearance of the main peak in the cathodic scan (wave E) and the appearance of the new peak (wave G) just next to the main peak may be attributed to the reduction of the product obtained in the first cycle characterized by the main wave to give another product which may be characterized by the new wave.

The decrease in the peak heights in the anodic scan, and the appearance of the catalytic wave are indications of the catalytic process taking place where the norfloxacin reactant (species θ) is formed again

according to the proposed mechanism:

$$O + ne \text{ -----> } R$$

$$R + ne \text{ -----> } P$$

$$P + Z \text{ -----> } O$$

in which Z is a nonreducible substance at a certain potential if it were present alone, causes the current obtained from the reduction of an electroactive substance O at that potential to increase by reacting with the product R to regenerate O or to form other substance that is reducible (6, 9). Thus, the type of the mechanism proposed is an EEC mechanism.

Also, the repetitive cyclic voltammograms of norfloxacin in 2M HCl showed some changes from the first cycle. The single wave exhibited by norfloxacin in this medium started to disappear by taking a shoulder shape with increasing the number of cycles. and the wave disappeared completely by cycle number five. This phenomenon may be ascribed to an EC mechanism, where the reduced product obtained from the main electrochemical-reduction process may be reacted again chemically in such a way to give another product which is electrochemically inactive or reduced at more negative potentials which are not accessible on the voltammogram.

Nalidixic acid did not show appreciable alterations with more cycles as in the case of norfloxacin (Fig. 4.13). The main change that occurred in its first cyclic voltammogram with more cycles is the appearance of the catalytic wave at -1.17V in the third cycle which showed an increase in size and shift to more negative potentials with the increasing number of cycles.

In the sixth cycle, the catalytic wave reached a potential position of -1.26V. In the cathodic scan, the three main peaks (waves 2, 4 and 5) decreased clearly in height in the second cycle; however, they were kept constant in the third cycle. When the catalytic process started taking place, the three main peaks started to increase gradually in height. This may be ascribed to the regeneration of the reactant (nalidixic acid) by means of the catalytic process. Thus, the mechanism proposed here is also a regeneration mechanism (6, 9) but different from the one proposed for norfloxacin,

$$O + ne \text{ -----> } R$$

$$R + Z \text{ -----> } O$$

where peak current given by the first reaction is directly proportional to the concentration of the substance Z.

4.7 Effect of Oxygen on the Cyclic Voltammograms of Norfloxacin and Nalidixic Acid

The effect of oxygen on the cyclic voltammograms of 10 ml of 0.1M LiCl base electrolyte without dearation was investigated. It was found that in the cathodic scan, oxygen shows two main peaks at -0.28V and at -1.08V and a shoulder for the first main peak at -0.16V. However, in the anodic scan oxygen gave rise to one peak at -0.08V as shown in Fig. 4.14, voltammogram A. The same solution of base electrolyte was then dearated for 4 min with nitrogen free oxygen and another voltammogram was recorded. The latter CV voltammogram did not show any peak with both scaning directions (cathodic and anodic), which means that no oxygen was found in the solution (Fig. 4.14, voltamogram B).

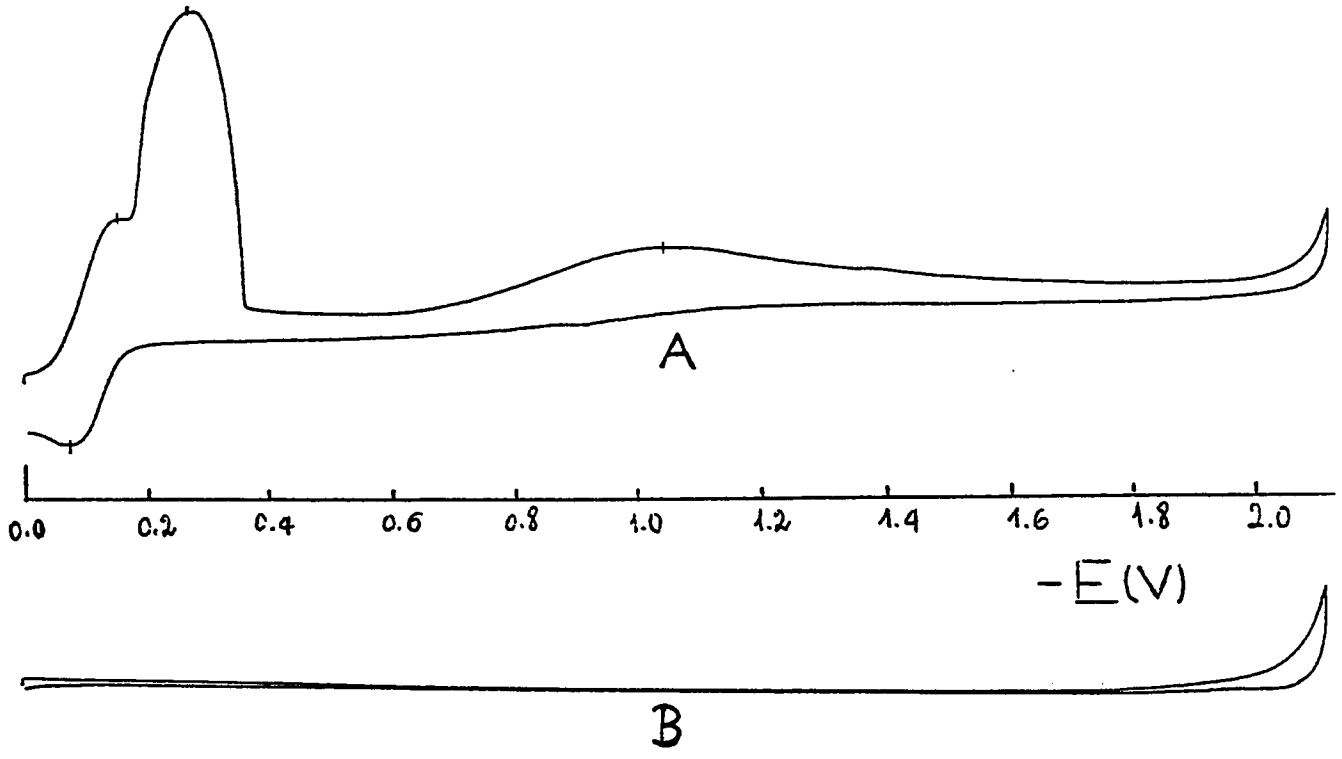


Fig 4.14 Cyclic voltammograms for 10 ml of 0.1M LiCl base electrolyte for the study of the effect of oxygen on the voltammograms of norfloxacin and nalidixic acid. (A) Without dearation (B) 4 min dearation with oxygen free nitrogen. The scan rate used was 50 mV/sec, and the current sensitivity was 2 μA.

These results, show that oxygen (O_2) is electrochemically active. It can be easily reduced to H_2O_2 and H_2O (46). For instance, the wave observed at -1.10V is ascribed to the reduction of oxygen to oxygen radical anion (superoxide) as in the reaction (47):

$$O_2 + e \rightleftharpoons O_2^-$$

and the peak current of the resulting wave increases as the rate of reduction increases.

From our previous studies on norfloxacin and nalidixic acid, we noted that both compounds reduce and give rise to peaks in the same potential region as oxygen. Thus, to avoid any overlapping between the peaks of oxygen and the peaks of the interested compounds deoxygenation is a necessary procedure in any electrochemical work on norfloxacin or nalidixic acid.

4.8 Determination of Norfloxacin in Noroxin Tablets by Linear Scan Voltammetry

Noroxin tablets were assayed by the external standards calibration method after grinding and dissolving in DMF according to the normal practice mentioned above. Two different scan rates were used in the determination procedure. A scan rate of 50 mV/sec was used in the first part and good results were obtained, as observed from Table 4.7. Table 4.7 shows good recoveries, an average of 100.57% recovery and a relative standard deviation, RSD, of 2.49% for the different base electrolytes

Table 4.7: Determination of norfloxacin in Noroxin tablets using the external standards calibration method. The main peak was used for analysis as indicated in the table below. Scan rates of 50 mV/sec or 100 mV/sec were used.

Base Electr. and Ep used (v)	Conc. range (ppm)	Corr. Coeff.	Slope (x 10^{-3}) (uA.L.mg^{-1})	Quantity added (ppm)	Quantity found (ppm)	Recovery %
I. V = 50 mV/sec						
CH_3COONa -1.48 / -1.54	31-292	0.9995	5.42	63.81 94.79 154.97 212.90 268.10	60.51 96.15 158.78 214.74 268.53	95.00 101.43 102.46 100.86 99.93 AVR: 99.94 R.S.D. 2.91
K_3 Citrate -1.54 / -1.57	31 - 292	0.9991	5.76	63.81 94.78 154.97 212.90 268.70	64.30 97.15 160.80 212.13 260.72	100.77 102.50 103.76 99.64 97.03 AVR: 100.74 R.S.D. 2.61
LiCl -1.56 / -1.60	31 - 292	0.9993	5.75	63.81 94.79 125.17 154.97 184.21 212.90 241.06 295.85	63.90 96.78 129.65 160.78 183.77 212.74 235.56 295.84	100.14 102.09 103.58 103.75 99.76 99.93 97.72 99.99 AVR: 100.89 R.S.D. 2.09
					AVERAGE R.S.D.	100.57 2.36

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Base Electr. and Ep used (v)	Conc. range (ppm)	Corr. Coeff.	Slope (x 10^{-3}) (uA.L.mg^{-1})	Quantity added (ppm)	Quantity found (ppm)	Recovery %
II. V = 100 mV/sec						
NaOAc -1.48 / -1.53	15 - 153	0.9999	7.87	32.22 63.81 94.78 125.16 154.97	31.16 61.68 92.70 123.23 152.25	96.71 96.67 97.81 98.50 98.24 AVR: 97.60 R.S.D. 0.85
K_3 Citrate -1.53 / -1.55	15 - 153	0.9993	8.58	32.22 63.81 94.78 125.16 154.97	31.61 64.72 94.74 123.60 150.07	98.11 101.43 99.96 98.75 96.81 AVR: 99.01 R.S.D. 1.77
$K_2B_4O_7$ -1.60 / -1.62	15 - 139	0.9994	11.36	32.22 63.81 79.37 94.78 125.16	30.60 63.93 77.93 92.54 121.83	95.03 100.19 98.20 97.64 97.34 AVR: 97.70 R.S.D. 1.85
KCl -1.50 / -1.55	15 - 153	0.9998	8.14	32.22 63.81 94.78 125.16 154.97	32.42 63.62 96.62 126.22 154.29	100.62 99.70 101.94 100.85 99.56 AVR: 100.53 R.S.D. 0.96
LiCl -1.55 / -1.58	15 - 153	0.9998	8.78	32.22 63.81 94.78 125.16 154.97	32.34 64.19 95.60 124.76 153.93	100.37 100.59 100.86 99.68 99.34 AVR: 100.17 R.S.D. 0.64
HCl (2M) -1.02 / -1.02	15 - 305	0.9996	2.20	------ because of	----- low sens.	---------
					AVERAGE R.S.D.	99.00 1.73

tested. However, when the scan rate of 100 mV/sec was used good results were obtained as shown in Table 4.7. An average of 99.0% recovery and a relative standard deviation of 1.73% were obtained for the different base electrolytes used. With both scans, the main peak used for analysis showed good shape and sensitivity, (an average of 4.64 $\mu A.L.mg^{-1}$ with 50 mV/sec and 8.95 $\mu A.L.mg^{-1}$ with 100 mV/sec). It is obvious that the sensitivity of the main peak increased with scan rate. However, norfloxacin in 2M HCl base electrolyte showed the lowest sensitivity, thus, it was not used in the determination of norfloxacin in Noroxin tablets. With both scan rates, the main peak appeared far from other peaks and base electrolyte discharge curve which may interfere with it. Thus, it is obvious that the drug excipients and the slight turbidity in the sample did not interfere in the determination procedure, and hence, did not affect the voltammetric behavior of norfloxacin.

The reproducibility of the linear scan voltammetric signal used for the determination of norfloxacin in Noroxin tablets was investigated in LiCl base electrolyte using both 50 and 100 mV/sec. Ten successive voltammograms of 5 x 10^{-5} M norfloxacin were recorded with a scan rate of 50 mV/sec as shown in Fig. 4.15 A. It was found that the peaks are very reproducible in which they have showed a relative standard deviation of 0.028% for n = 10. Another ten successive voltammograms of 1 x 10^{-4} M norfloxacin were recorded with a scan rate of 100 mV/sec. They showed a good reproducibility with a relative standard deviation of 0.070% for n = 10 (Fig. 15, B). Thus, it can be concluded that the system is very reproducible and the present method is highly recommended for the quantitative determination of norfloxacin in formulated tablets.

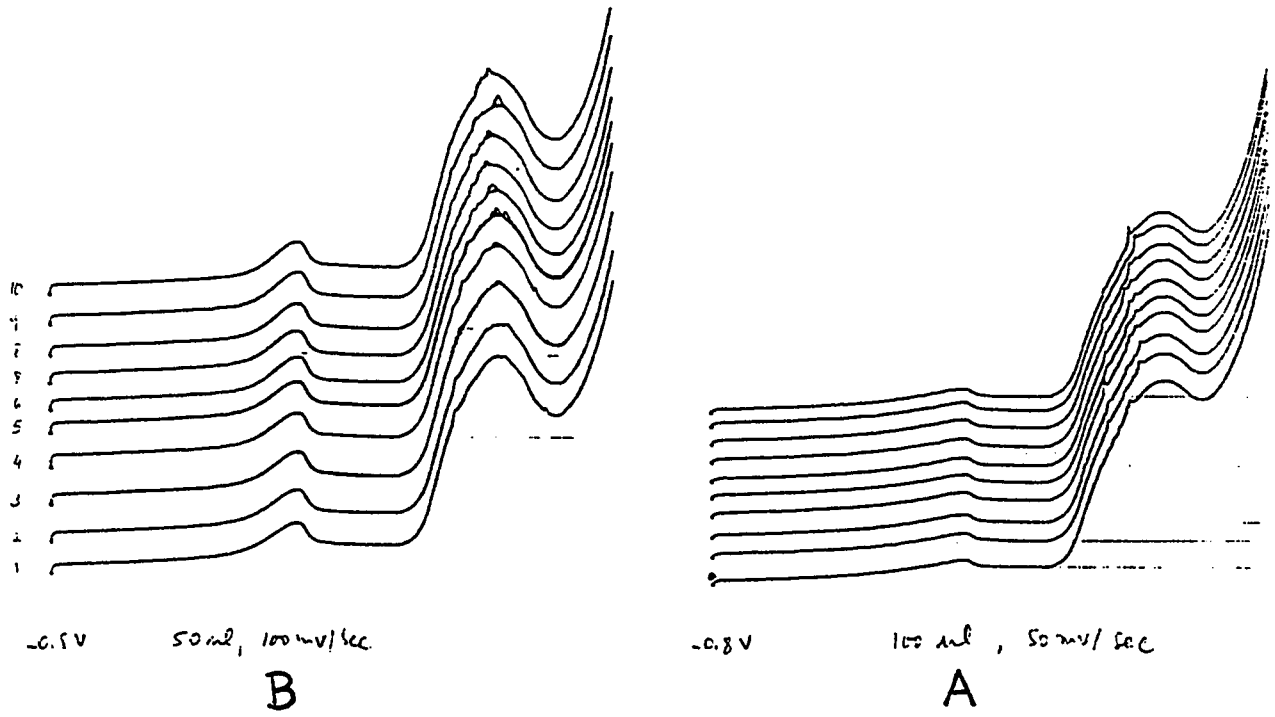

Fig 4.15 Reproducibility of the linear scan voltammetric signal. Ten successive voltammograms of 1×10^{-4}M norfloxacin in 0.1M LiCl were recorded with scan rates (A) 50 mV/sec and (B) 100 mV/sec. The current sensitivity was 2 μA.

4.9 Determination of Nalidixic Acid in Negram Tablets by the Linear Scan Voltammetry Technique

Negram tablets were also assayed by the external standards calibration method after their preparation in DMF according to the normal practice mentioned above. The three main peaks of nalidixic acid (Fig. 4.16) were used in the analytical study as reported in Tables 4.8 and 4.9. As with norfloxacin, two different scan rates were used in the determination procedure. A scan rate of 50 mV/sec was used in the first part of the study. Good results were obtained using the three peaks, as shown in Table 4.8. Table 4.8 shows good recoveries using the three peaks namely, peak 1, peak 2, peak 3 (Fig. 4.16 and Tables 4.8 and 4.9), and their summation (peak 1 + peak 2 + peak 3). Average recoveries of 101.66%, 101.12%, 103.59% and 101.37% with relative standard deviations RSD, of 1.87%, 2.14%, 1.84% and 1.61% were obtained respectively, for the different base electrolytes used.

However, when the scan rate of 100 mV/sec was used good results were obtained using the three peaks (peak 1, peak 2, peak 3) and their summation (peak 1 + peak 2, peak 1 + peak 2 + peak 3) as observed from Table 4.9. Average recoveries of 100.58%, 101.47%, 102.76%, 100.01% and 101.68% with relative standard deviation, RSD, of 1.95%, 2.28%, 1.78%, 2.08% and 2.34% were obtained respectively, for the different base electrolytes tested. Also, with both scans, the peaks used for analysis showed good shapes and sensitivities (an average of 6.28, 6.33 and 10.5 $\mu A.L.mg^{-1}$ respectively with scan rate of 50mV/sec and 10.45, 11.16 and

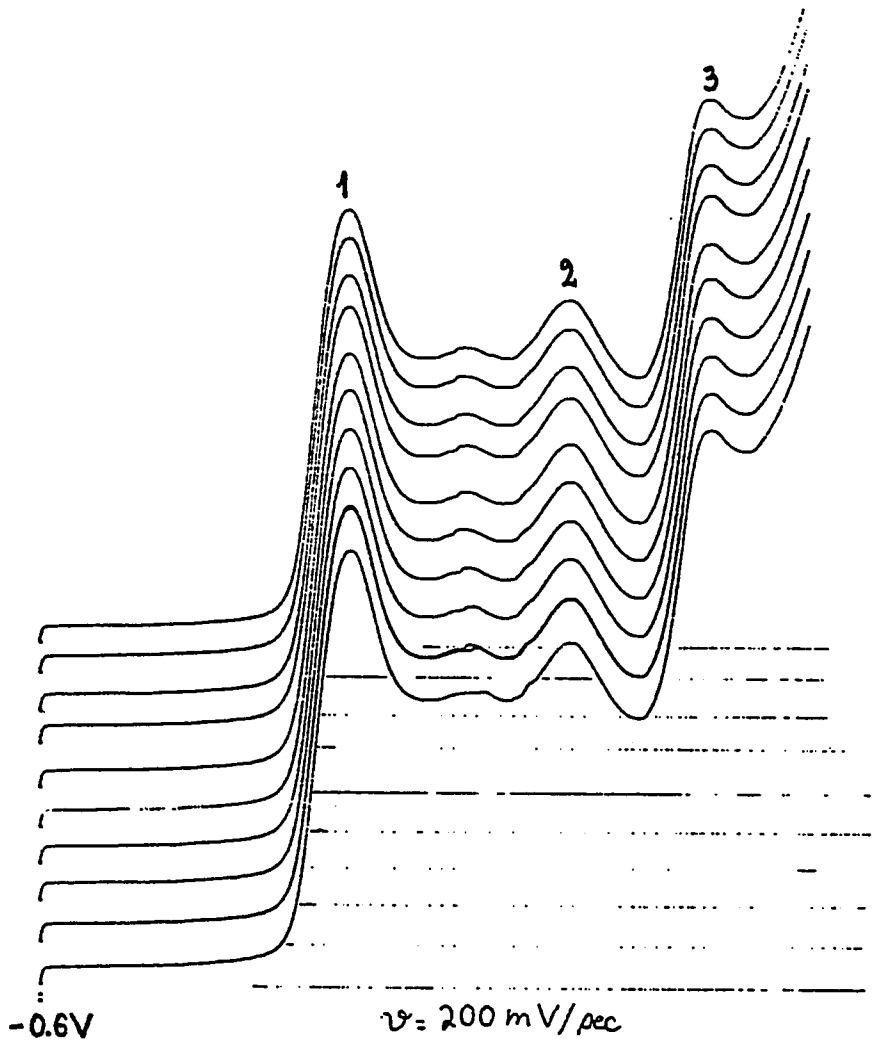

Fig 4.16 Reproducibility of the linear scan voltammetric signal. Ten successive voltammograms of 1×10^{-4}M nalidixic acid in 0.1M LiCl were recorded. The scan rate used was 200 mV/sec. The current sensitivity was 2 μA.

Table 4.8: Determination of nalidixic acid in Negram tablets using the external standards calibration method. The three main peaks were used for analysis as indicated by the table below. The scan rate used was 50 mV/sec.

Base Electr. and Ep used (v)	Conc. range (ppm)	Corr. Coeff.	Slope (x 10^{-3}) ($\mu A.L.mg^{-1}$)	Quantity added (ppm)	Quantity found (ppm)	Recovery %
I. Peak # 1						
NaOAc -1.36 / -1.26	23 - 213	0.9999	7.81	23.26 46.06 68.43 111.87 153.69 193.97	23.35 46.02 69.07 112.57 155.93 195.75	100.39 99.92 100.94 100.63 101.46 100.92 AVR: 100.71 R.S.D. 0.53
K_3 Citrate -1.36 / -1.36	23 - 213	0.9998	4.26	46.06 68.43 90.36 111.87 153.69 193.97	45.88 68.06 92.11 115.26 161.53 196.74	99.61 99.47 101.94 103.03 105.11 101.42 AVR: 101.76 R.S.D. 2.14
LiCl -1.10 / -1.15	23 - 133	0.9996	6.77	23.26 46.06 68.43 90.36 111.87	23.60 45.99 70.11 95.68 116.03	101.46 99.85 102.46 105.88 103.72 AVR: 102.67 R.S.D. 2.28
					AVERAGE R.S.D.	101.66 1.87

....Contd./2,

Base Electr. and Ep used (v)	Conc. range (ppm)	Corr. Coeff.	Slope (x 10^{-3}) ($\mu A.L.mg^{-1}$)	Quantity added (ppm)	Quantity found (ppm)	Recovery %
II. Peak # 2						
NaOAc -1.52 / -1.43	23 - 213	0.9998	8.42	23.26 46.06 68.43 111.87 153.69 193.97	23.13 46.07 69.19 115.09 157.18 194.60	99.44 100.01 101.12 102.88 102.27 100.32 AVR: 101.01 R.S.D. 1.34
K_3 Citrate -1.56 / -1.49	23 - 213	0.9990	3.96	46.06 68.43 90.36 111.87 153.69 193.97	48.71 69.97 90.73 111.54 151.04 194.97	105.74 102.25 100.41 99.71 98.28 100.51 AVR: 101.15 R.S.D. 2.59
LiCl -1.52 / -1.52	23 - 133	0.9998	6.62	23.26 46.06 68.43 111.87	22.87 45.68 70.68 116.72	98.31 99.17 103.20 104.33 AVR: 101.25 R.S.D. 2.96
					AVERAGE R.S.D.	101.12 2.14

....Contd./3,

Base Electr. and Ep used (v)	Conc. range (ppm)	Corr. Coeff.	Slope (x 10^{-3}) ($\mu A.L.mg^{-1}$)	Quantity added (ppm)	Quantity found (ppm)	Recovery %
III. Peak # 3						
NaOAc -1.78 / -1.78	23 - 133	0.9999	11.99	PK # 3 of the	unknowns was	not recorded n o t
K_3 Citrate -1.76 / -1/76	23 - 133	0.9994	6.57	"	"	"
LiCl -1.72 / -1.72	23 - 133	0.9998	12.94	23.26 46.06 68.43 90.36 111.87	23.43 47.88 71.36 95.60 115.60	100.73 103.94 104.17 105.80 103.33 AVR: 103.59 R.S.D. 1.84
IV. Peak addition						
K_3 Citrate PK 1 + PK 2 (potentials were previously)	23 - 213	0.9996	8.07	46.06 68.43 90.36 111.87 153.69 193.97	45.65 69.41 93.27 115.76 155.75 194.14	99.11 101.44 103.22 103.03 101.34 100.08 AVG: 101.37 R.S.D. 1.61
K_3 Citrate PK 1 + PK2 + PK3	23 - 133	0.9997	12.40			

Table 4.9: Determination of nalidixic acid in Negram tablets using the external standards calibration method. The three main peaks were used for analysis as indicated in the table below. The scan rate used was 100 mV/sec.

Base Electr. and Ep used (v)	Conc. range (ppm)	Corr. Coeff.	Slope (x 10^{-3}) ($\mu A.L.mg^{-1}$)	Quantity added (ppm)	Quantity found (ppm)	Recovery %
I. Peak # 1						
NaOAc -1.37 / -1.29	11 - 112	0.9998	10.74	11.68 23.26 46.06 68.43 90.36 111.87	11.09 23.18 46.85 69.59 90.87 113.25	95.00 99.70 101.71 101.70 100.56 101.21 AVR: 99.98 R.S.D. 2.56
K_3 Citrate -1.36 / -1.36	11 - 112	0.9991	5.24	11.68 23.26	12.10 23.29	103.60 100.13 AVR: 101.86 R.S.D. 2.45
KCl -1.15/-1.12/-1.16	11 - 112	0.9995	8.89	11.68 23.26 46.22 68.43 90.36 111.87	11.67 23.68 46.22 68.81 92.73 116.20	99.97 101.80 100.34 100.56 102.62 103.87 AVR: 101.52 R.S.D. 1.52

....Contd./2,

Base Electr. and Ep used (v)	Conc. range (ppm)	Corr. Coeff.	Slope (x 10^{-3}) ($\mu A.L.mg^{-1}$)	Quantity added (ppm)	Quantity found (ppm)	Recovery %
LiCl 1.16/-1.11/-1.16	11 - 90	0.9998	9.24	11.68 23.26 46.06 57.30 68.43 90.36	11.35 24.01 46.23 56.92 69.21 92.53	97.17 103.22 100.36 99.35 101.15 102.41 AVR: 100.61 R.S.D. 2.18
HCl (1M) -0.79 / -0.86	11 - 90	0.9999	18.15	23.26 46.06 57.30 68.43 90.36	22.97 45.87 56.96 68.45 90.61	98.75 99.60 99.41 199.04 100.27 AVR: 99.61 R.S.D. 0.59
					AVERAGE R.S.D.	100.58 1.95

....Contd./3,

Base Electr. and Ep used (v)	Conc. range (ppm)	Corr. Coeff.	Slope (x 10^{-3}) (μA.L.mg^{-1})	Quantity added (ppm)	Quantity found (ppm)	Recovery %
II. Peak # 2						
NaOAc -1.54 / -1.45	11 - 112	0.9997	10.84	23.26 46.06 68.43 90.36 111.87	22.56 47.47 69.88 91.59 114.72	97.00 103.05 102.13 101.36 102.55 AVR: 101.22 R.S.D. 2.44
K_3 Citrate -1.58 / -1.51	11 - 112	0.9990	6.66	11.68 46.06 68.43 90.36 111.87	11.98 45.93 71.92 92.62 110.96	102.48 99.73 105.11 102.50 99.20 AVR: 101.80 R.S.D. 2.40
$K_2B_4O_7$ -1.61 / -1.61	11 - 90	0.9981	9.98	11.68 23.26 46.06 57.30 68.43 79.44	11.10 23.64 48.79 58.26 68.27 80.16	95.03 101.63 105.91 101.67 99.77 100.90 AVR: 100.82 R.S.D. 3.52
KCl -1.50 / -1.50	11 - 112	0.0002	8.25	11.68 23.26 46.06 68.43 90.36 111.87	11.82 23.46 47.81 69.88 91.20 112.03	101.11 100.86 103.79 102.13 100.93 100.14 AVR: R.S.D. 1.29

....Contd./4,

Base Electr. and Ep used (v)	Conc. range (ppm)	Corr. Coeff.	Slope (x 10^{-3}) ($\mu A.L.mg^{-1}$)	Quantity added (ppm)	Quantity found (ppm)	Recovery %
LiCl -1.50 / 1.50	11 - 90	0.9994	8.94	23.26 46.06 57.30 68.43 90.36	22.26 46.66 58.05 69.65 91.56	95.70 101.29 101.31 101.79 101.33 AVR: 100.28 R.S.D. 2.57
HCl (1M) -1.06 / -1.06	11 - 80	0.9997	22.30	11.68 23.26 46.06 57.30 68.43	11.43 23.64 48.32 59.77 71.26	97.80 101.63 104.90 104.31 104.14 AVR: 102.56 R.S.D. 2.94
					AVERAGE R.S.D.	101.47 2.28

....Contd./5,

Base Electr. and Ep used (v)	Conc. range (ppm)	Corr. Coeff.	Slope (x 10^{-3}) (μA.L.mg^{-1})	Quantity added (ppm)	Quantity found (ppm)	Recovery %
II. Peak # 3						
NaOAc -1.78 / 1.78	11 - 112	0.9998	15.84	11.68 23.26 46.06 68.43 90.36	12.00 23.67 48.36 71.42 94.79	102.65 101.76 105.00 104.37 104.90 AVR: 103.74 R.S.D. 1.45
K_3 Citrate -1.76 / 1.76	11 - 101	0.9993	8.59	46.06 68.43 90.36	47.90 70.62 93.35	103.98 103.21 103.31 AVR: 103.50 R.S.D. 0.42
$K_2B_4O_7$ -1.75 / -1.75	11 - 90	0.9999	20.83	11.68 23.26 46.06 57.30 68.43 79.44	11.24 23.55 48.10 58.31 69.65 80.24	96.27 101.25 104.42 101.76 101.79 101.00 AVR: 101.08 R.S.D. 2.66
KCl -1.76 / 1.76	11 - 101	0.9999	10.88	23.26 46.06 68.43 90.36	24.00 45.96 70.90 94.59	103.00 99.78 103.62 104.68 AVR: 102.77 R.S.D. 2.11
LiCl -1.72 / -1.72	11 - 90	0.9995	16.30	46.06 57.30 68.43 90.36	45.60 57.74 69.94 92.16	99.00 100.77 102.21 102.003 AVR: 100.99 R.S.D. 1.47

....Contd./6,

Base Electr. and Ep used (v)	Conc. range (ppm)	Corr. Coeff.	Slope (x 10^{-3}) ($\mu A.L.mg^{-1}$)	Quantity added (ppm)	Quantity found (ppm)	Recovery %
IV. Peak addition K_3 Citrate						
PKs 1 + 2 (potentials were mentioned previously	11- 112	0.9994	12.47	23.26 46.06 68.43 90.36 111.87	22.42 46.60 69.42 91.14 112.09	96.39 101.16 101.45 100.86 100.20 AVR: 100.01 R.S.D. 2.08
Pks 1 + 2 + 3	11 - 101	0.9999	16.99	11.68 23.26 46.06 68.43 90.36	12.10 24.40 47.17 70.80 92.58	103.50 104.90 102.40 103.47 102.46 AVR: 103.35 R.S.D. 1.02
					AVERAGE R.S.D.	101.68 2.34

14.50 μA.L.mg^{-1} with 100 mV/sec). Thus, we note that, the sensitivity of the main peaks increases with scan rate as in the case of norfloxacin. In contrary to norfloxacin, nalidixic acid gave the best sensitivity (18.14 μA.L.mg^{-1} for the main peak and 22.30 μA.L.mg^{-1} for the following peak) in 1M HCl base electrolyte, hence it was also used for the determination of nalidixic acid in Negram tablets. With both scan rates (50 and 100 mV/sec) the three main peaks appeared far from any other peak or base electrolyte discharge curve which may interfere with them in any qualitative or quantitative analytical work. Hence, it is also obvious from the results, that the drug excipients and the slight turbidity in the sample did not interfere in the determination procedure, and thus, did not affect the polarographic behavior of nalidixic acid.

The reproducibility of the three main peaks used in the determination of nalidixic acid in Negram tablets was also investigated in LiCl base electrolyte using a scan rate of 200 mV/sec. Ten successive voltammograms of 1 x 10^{-4} M nalidixic acid were recorded with a scan rate of 200 mV/sec as shown in Fig. 4.16. It was found that the three main peaks were very reproducible and showed relative standard devisations, RSD, of 0.033%, 0.027% and 0.022%, for peaks 1, 2, and 3 respectively (n = 10). The last peak at -1.85V (Table 4.3) also showed a good reproducibility with an RSD of 0.076% for n = 10, but it was not used in the analytical determination because it is very close to the base electrolyte discharge curve. Finally, we conclude that this system is very reproducible and the present method is also highly recommended for the quantitative determination of norfloxacin in commercial tablets.

CHAPTER FIVE

CONCLUSIONS

The dc and dp polarographic reduction of the Antibacterial drug norfloxacin has been studied in various base electrolytes at different pH values and in the presence of dimethyl formamide. Only one reduction wave in the range of -0.095V to -1.02V was observed in strongly acidic medium (> 0.1M HCl). Using base electrolyte of a pH ≥ 7.5, two well defined irreversible waves were observed in the ranges of -1.42V to -1.58V (wave C) and -1.74V to -1.85V (wave D). The position of these dp polarographic waves within these ranges was dependent on the pH of the medium and norfloxacin concentration. At pH > 10 only wave D was observed, but all waves disappeared completely in 0.1 M NaOH. In addition, two ill defined waves (adsorptive waves) appeared in the range of -0.06V to -0.42V within the pH range of 6.5 to 8.5 for norfloxacin concentrations > 5×10^{-5} M.

The single wave at about -1.02V in 2M HCl and wave C in the other base electrolytes showed a useful rectilinear relationship between concentrations and wave heights in the range of 32 to > 560 ppm norfloxacin. The two dp waves have been utilized for determination of norfloxacin in Noroxin tablets with good percent recoveries and small relative standard deviation using both methods, the external standards calibration method and the standard addition method. A concentration

of ~1 ppm is detectable. Norfloxacin was also studied in the presence of 45.56 ppm of nalidixic acid as interferent using the dpp technique. Useful rectilinear curves were obtained in the range 0 - 121 ppm using NaOAc and K_3citrate as base electrolytes. Good recoveries with small R.S.D. were obtained using the external standards calibration method.

Polarograpic behavior of norfloxacin has been compared to that of nalidixic acid which has been also determined successfully in Negram tablets as the individual component and in the presence of norfloxacin as an interferent.

An analytical approach to equimixture of norfloxacin and nalidixic acid was conducted. The first peak of nalidixic acid in NaOAc at -1.32V and in 2M HCl at -0.78V were used for the calibration curves construction. Good linearities were obtained in the range of 0 - 270 ppm in NaOAc with a correlation coefficient of 0.9994 and over a range of 0 - 200 ppm with a correlation coefficient of 0.9993 in 2M HCl. It has been concluded that these two peaks belong to nalidixic acid only and thus, were used for its determination quantitatively and qualitatively in the presence of norfloxacin with minimum interferences.

Based on the dc and dpp study of norfloxacin, a proposed mechanism has been suggested for its reduction at the DME. Norfloxacin is expected to exist in aqueous media in different forms (protonated and nonprotonated due to acid-base equilibria) depending on the pH of the medium. At intermediate pH (pH ~ 7), it is expected to exist as a Zwitterion, HA_Z, a neutral molecule, HA_N and a small proportion of the conjugate acid H_2A^+.

However, in a basic medium (pH ≥ 10) the conjugate base A^- predominates. Meanwhile, in a strongly acidic medium (2M HCl, for example) the diprotonated species H_3A^{2+} would be the major one existing in the solution.

Based on the dp polarograms in base electrolytes of wide pH range, appearance and disappearance of waves according to pH values of the solutions and the proposed equilibria, the wave at -1.02V in strongly acidic media of HCl solutions and waves C and D in the intermediate and basic media could be attributed to one-electron reduction steps of the species, H_3A^{2+}, HA_Z and A^- respectively. This reduction will produce the radical products: H_3A^+, HA_Z^- and A^{2-}. These radicals may deactivate by dimerization, disproportionation or reaction with the solvent. It may be also suggested that the radicals may undergo further one-electron reduction step producing waves shifted to potentials more negative than the discharge potential of the base electrolyte. The absence of waves related to the species, HA_N and H_2A^+ may be ascribed to their limited proportions in solutions at an intermediate pH range.

Norfloxacin was also studied using the differential pulse adsorptive stripping voltammetry. The method described, demonstrates the coupling of catalytic and adsorption processes which constitute the basis for an ultrasensitive and simple approach to the determination of ultratrace levels of norfloxacin. The catalytic - adsorptive accumulation results in a 2-3 orders of magnitude lowering of the detection limit where other physico-chemical methods are often no longer useful. Because of the inherent sensitivity of the method, significant dilutions and short preconcentration times (15s - 20s) could be used. The adsorptive peak at -1.06V was used

for analysis since it is always the best well defined one. It gave reasonable linearities within rectilinear ranges from 0.3206 to 400 ptt (1.00×10^{-12} M to 1.25×10^{-9} M) and from 0.2570 to 60 ppb (8.05×10^{-10} M to 1.88×10^{-7} M) norfloxacin. This peak was the most extensively used to test the validity of the method for the determination of the drug in commercial tablets (Noroxin). Using the optimum conditions obtained for the different methodological and instrumental variables for the accumulation of norfloxacin, resulted in extremely low detection limit of 0.321 ppt (1.00×10^{-12} M) following an accumulation period of 4 min.

The major disadvantage faced, is the adsorption interferences from the base electrolytes used which needs thorough purification, and also, may be from the drug excipients (turbidity) which needs filtration of the Noroxin sample solutions prior to analysis.

Further advantage is the relatively inexpensive instrumentation, as a classical polarographic arrangement can be employed.

The linear sweep and cyclic voltammetric behavior of norfloxacin and nalidixic acid were investigated. Using cyclic voltammetry, the two main reduction waves of norfloxacin were confirmed to be irreversible. the main peak in the range -1.48V to -1.63V was found to be adsorption controlled with small scan rates ($\leq$ 20 mV/sec) and diffusion controlled at higher scan rates. However, the other one at -1.77V to -1.80V was found to be diffusion controlled. The other smaller peaks showed different degree of reversibility depending on the scan rate, and they were found to be adsorption controlled.

The proposed mechanism using dc and dpp was found to be incomplete. Norfloxacin is found to be reduced in one-electron step in strongly acidic and strongly alkaline solutions to give the radical species H_3A^+ and A^{2-}. However, it is reduced in 2 one-electron steps in slightly acidic to slightly alkaline solutions (pH 6.5-8.0) where a dihydrocompound is obtained as the product by introducing two hydrogens on the ethylenic bond in the azinone ring which is the polarographically active center. The reduction processes of nalidixic acid are found to be completely irreversible. The first wave to the most positive potential was found to be of adsorption nature and the other waves were found to be diffusion controlled processes.

The proposed mechanism for the electroreduction of nalidixic acid at the DME using dc polarography technique was confirmed by using cyclic voltammetry which is a more versatile technique. Each step of the mechanism had a clear evidence. Nalidixic acid is found to be reduced in 2 one-electron steps in strongly acidic media and in slightly acidic to slightly alkaline solutions. A dynamic process is involved in the electroreduction process, where protonation is more likely to occur on the ethylenic bond (electroactive center) in the azinone ring which is also reduced by introducing two hydrogens to obtain the dihydroderivative compound which is the final product. However, nalidixic acid is reduced in one-electron only in highly alkaline medium to give the radical species A^{2-} which may be deactivated or may undergo further one-electron reduction step producing a wave to more negative potential.

A plot of i_p vs. pH was obtained, and the main wave showed a dissociation curve with an inflection point at pH 6.2. This pH is considered to be the polarographic pK_a (for this wave) for nalidixic acid which is found to be in good agreement with the value 6.02 - 6.11 mentioned in the literature. Such agreement implies an acid-base equilibrium established slowly when compared with the time window (3 sec) of a polarographic measurement.

Repetitive cyclic voltammograms of norfloxacin and nalidixic acid were investigated. Both compounds showed catalytic behavior with scan rates of 50 mV/sec and smaller at intermediate pH (pH 7.0 - 8.0). The catalytic waves were found to increase in size with the number of cycles (time), which are indicative of a catalytic process taking place slowly were the reactant (norfloxacin or nalidixic acid) is regenerated and then reduced at the specific potential by showing a cathodic current which is known as a regenerative current in the anodic scan.

Moreover, the effect of oxygen on the cyclic voltammograms of norfloxacin and nalidixic acid was also investigated, and it was found that to avoid any overlapping between the peaks of oxygen and the peaks of the interested compounds, deoxygenation is a necessary step in any voltammetric work on norfloxacin or nalidixic acid.

Norfloxacin and nalidixic acid were also determined in Noroxin and Negram tablets, respectively, using the linear scan voltammetry. The resproducibility of the linear scan voltammetric signals used for the determination were investigated in LiCl base electrolyte using both 50 and

100 mV/sec and showed excellent reproducibility. Good recoveries were obtained with small R.S.D. values with a detection limit of ~1 ppm. The method is easy, quick, economic, and gave good results. Thus, it is highly recommended to be used for the quantitative determination of norfloxacin and nalidixic acid in formulated tablets.

Suggestions for Further Work

To study the other compounds of the 4-quinolone family for the objectives:

i. To develop and confirm a general mechanism for their reduction process.

ii. To develop economic, easy and quick procedures for their determination as individuals and in mixtures.

iii. To study the possibility of complex formation with transition metals and their stability.

iv. To develop ion (membrane) selective electrodes for their quantitative assay.

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